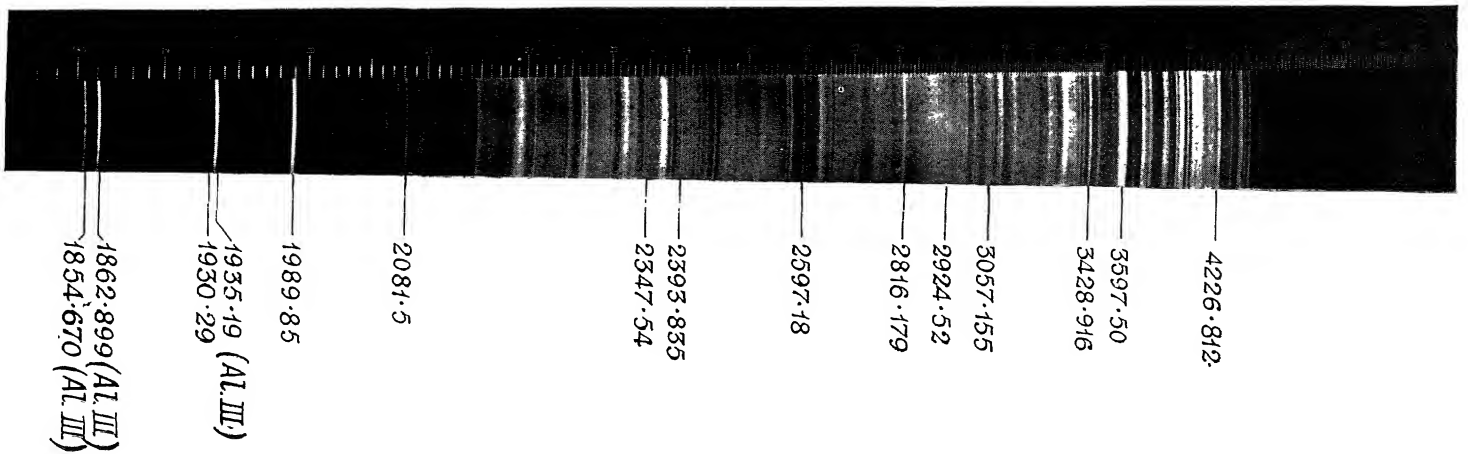
SPARK SPECTRUM OF ALUMINIUM Al. II and Al. III.

Taken on Hilger Quartz Spectograph E 370.

Exposure = 2 seconds on Hilger Schumann Plate.
Slit Width = 0·05 mm.



All values except those marked Al III are from Paschen *Ann der Phys*, **71**, 537 (1923)
The Al III values are from Paschen, *Ann der Phys*, **71**, 141 (1923) All values are in I A.

THE PRACTICE OF

Absorption Spectrophotometry

WITH

HILGER INSTRUMENTS

An introduction to the Theory of the Subject, and Guide to the Technique of Absorption Measurement in the Visible, Ultra-violet, and Infra-red Regions of the Spectrum.

BY

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AND

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PREFACE

THE theory of absorption and the applications of absorption spectrophotometry have made such rapid development in the last few years that, in spite of the fact that the first edition of this book was published as recently as 1932, it had already become desirable a year ago that the whole subject should be reviewed afresh to assist the publishers in their development of spectrographic equipment. The work then undertaken soon revealed a need for the preparation of a second edition of this book, to provide guidance for those wishing to commence spectrophotometric work, or to extend it into fields not yet familiar to them.

Part I of the present edition therefore contains a large amount of entirely new matter contributed by one of us (C. B. A.), the original author (F. T.) having now confined his attention chiefly to Part II, in connection with which he wishes to acknowledge the help received from the members of the staff of Adam Hilger, Limited.

The first edition had its origin in two articles published in *Chemistry and Industry*.[1] The writer renews his acknowledgment of his indebtedness to the publishers of that Journal for permitting him to embody with slight revision the substance of those articles, some of which is retained in the present edition.

Thanks are also due to the Physical Society for permission to include much of the substance of two papers,[2] and to Professor R. Mecke of Heidelberg, and to Dr. F. P. Bowden of Cambridge, for permission to reproduce original photographs of spectra, as well as to the Director of the Bureau of Standards for permission to publish Fig. 14 and to the publishers of the *Zeitschrift für Physik* for permission to reproduce Fig. 15.

The note on "The absorption of diffusing media," specially written for the book by Mr. O. W. Pineo, sets forth new ideas which may become of great importance in the textile industries.

Finally, we are indebted to Dr. F. Simeon, Chief Physicist of Adam Hilger, Limited, and Mr. L. J. Spencer for reading the proofs and for valuable suggestions, and to Mr. T. L. Tippell for the preparation of the index.

F. TWYMAN.
C. B. ALLSOPP.

[1] "Absorption Photometry and some of its Applications to Chemistry," F. Twyman, *Chemistry and Industry*, Vol. XLIX, Nos. 26, 27, 28, 1930.

[2] "Conditions for Securing Accuracy in Spectrophotometry," Twyman and Lothian, *Proc. Phys. Soc.* **45**, Part 5, No 250, September 1933.
"Spekker Photometer for Ultra-violet Spectrophotometry," F. Twyman, *Trans. Opt. Soc.* **33**, No. 1, 1931 and 1932.

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ABSORPTION SPECTRA AND THEIR APPLICATIONS

PART II

THE TECHNIQUE OF ABSORPTION SPECTROSCOPY

WAVE-LENGTHS AND WAVE-NUMBERS[1]

The position of a line in the spectrum is most generally indicated by the wave-length (λ) of the vibration which produces it. The unit of wave-length is the Ångström Unit, or " angstrom," as it is now called. It was intended to equal 10^{-10} metre, and is accordingly often called the " tenth-metre." It is also equivalent to 10^{-8} cm., or $0{\cdot}0001\mu$, where μ is the micron or thousandth of a millimetre. Wave-lengths in the visible spectrum range from about 3900 A to 7600 A (A being the modern abbreviation for the angstrom). For the long waves in the infra-red, however, μ is often taken as the unit, so that λ12500 A, for example, would be indicated by $1{\cdot}25\mu$.

The wave-length scale was introduced by Ångström in 1869, and until about the year 1900, wave-lengths were referred to his determinations as standards. In the course of time, however, they have been measured with increasing accuracy, and the latest measurements, based on interferometer determinations, are indicated by the letters I.A. (International Angstroms).

In connection with spectral series, whether of emission or of absorption spectra, it becomes important to specify the positions of lines either in "oscillation frequencies" or by " wave-numbers." The most fundamental figures are the oscillation frequencies, since these are not changed when the medium is changed. But the determination of frequency requires an exact knowledge of the velocity of light, and it is more convenient to use the wave-number, or number of waves per centimetre ; thus

$$\text{Wave-number} = 10^8/\lambda \text{ in angstroms.}$$

The word " fresnel " is sometimes used to denote the unit of frequency ;

$$1 \text{ fresnel} = 10^{12} \text{ vibrations per second.}$$

USEFUL PHYSICAL CONSTANTS.

Planck's Constant,	$h = 6{\cdot}542 \times 10^{-27}$ erg-secs.
Electronic Charge,	$e = 4{\cdot}7668 \times 10^{-10}$ e.s.u.
Electronic Mass,	$m = 9{\cdot}04 \times 10^{-28}$ gm.
1 Volt-Electron	$= 1{\cdot}59 \times 10^{-12}$ ergs.
Velocity of light,	$c = 3 \times 10^{10}$ cm per sec.
Avogadro's Number,	$N = 6{\cdot}062 \times 10^{23}$ per gm. molecule.
Mechanical Equivalent of Heat,	$J = 4{\cdot}185 \times 10^{7}$ ergs per cal.

[1] Abstracted from A. Fowler, *Report on Series in Line Spectra* (Physical Society of London, 1922).

PART I—ABSORPTION SPECTRA AND THEIR APPLICATIONS

CHAPTER I

THE NATURE OF ABSORPTION

WHEN light passes through any homogeneous transparent medium it emerges diminished in energy. Part of the light may be scattered at the surface, part scattered in the interior, and part regularly reflected at the surfaces. The rest of the light which is lost is said to be absorbed. It may be transformed into heat, or into fluorescent or phosphorescent light of wave-lengths differing from its own, as in the familiar behaviour of fluorescein solutions or the luminous paints employed on watch dials, or it may cause chemical ("photochemical") action, such as the reaction between hydrogen and chlorine induced by sunlight, or the recent production of vitamin-D from ergosterol under irradiation, observed by Heilbron and his co-workers.

The absorbed light is of great interest to chemists. The study of photochemical reactions has thrown light on many problems, and, together with investigations of the fluorescence which often accompanies photochemical changes, it can give valuable information as to the manner in which a molecule breaks up and the energy changes involved. An exact quantitative knowledge of the primary absorption process is thus fundamental [1]; but it is also of value in other directions. In a qualitative way, such knowledge has been employed for a long time. Whenever, in the course of his work, a chemist has occasion to observe a colour, and to draw a deduction from that observation, he is utilising a form of absorption spectroscopy. In this way, colorimetric tests are applied in analysis, both qualitatively and quantitatively, and have provided a means, even if crude, of following the progress of chemical reactions. Some colorimetric tests, however, can be made extremely sensitive, and they can then be used for accurate determinations, as in the case of the Nessler reagent employed in water analysis. When, therefore, in place of the eye we substitute a quartz spectrograph, which exposes to observation an additional spectral range considerably greater than that for which the eye is sensitive, and add means of measuring the proportion of radiation of each wave-length which is absorbed, it is not surprising that we arrive at a

[1] See Style, "*Photochemistry*" (1930).

very powerful and precise method of discriminating between different substances, estimating the strengths of their solutions and their purity, and of observing the rates of chemical changes. Some of these applications of absorption spectrophotometry will be described in more detail in a later chapter.

Some of the advantages of making observations of absorption in the ultra-violet with quartz apparatus are illustrated in Figs. 1, 2, 3, 4 and 5. Fig. 1 shows the difference in transparency of various types of optical glass, as compared with that of quartz, in a thickness of 1 cm., which can be seen to be completely transparent down to the limit of the aluminium spark[1] spectrum, whereas glass is opaque to wave-lengths below 3100 A for crown glass and below 3500 A for dense flint glass, although a special "ultra-violet" crown glass transmits down to 3000 A, transmitting 25% of the radiation at 3100 A. Most quartz is transparent, in a thickness of 1 cm., to radiation of wave-length 2000 A, and some specimens will transmit the 1854 A line of the aluminium spark spectrum. Beyond this wave-length, however, both quartz and air absorb strongly, and it is necessary to work with fluorspar optical systems in vacuo.[2]

Fig. 2 compares the transparencies to ultra-violet light of four liquids commonly used as solvents in absorption spectrum work, namely water, ethyl alcohol C_2H_5OH, *cyclo*hexane C_6H_{12}, and carbon tetrachloride CCl_4. The light source employed for these photographs was a hydrogen discharge tube, giving a continuous spectrum over a very wide range of wave-lengths. The figure shows that, while all four of these liquids are transparent over a wide region of the spectrum, they begin to cut off the light at different short wave-length limits. The actual wave-lengths are set out in Table II on page 67, Chapter V. With a more powerful light-source, such as the tungsten-steel spark, it is found that the liquids are still transparent to shorter wave-lengths (Fig. 3).

Many substances absorb so strongly at short wave-lengths that except in very high dilutions, they appear to be completely opaque in this way beyond a certain limit. Others, on the contrary, exhibit bands which are confined to a narrow range in the spectrum. This is illustrated in Fig. 4. The third of the photographs was taken, using an iron arc, through a film of Canada balsam 0·1 mm. thick. It transmits only to 2590 A. Canada balsam is used for cementing together parts of optical apparatus,

[1] A reproduction of the aluminium spark spectrum, on which the principal lines whose wave-lengths have been reliably measured are marked, will also be found facing the title page. This spectrum is included here on account of its great value in spectrophotometry at short wave-lengths, and because reproductions are inaccessible in the literature.

[2] Or with vacuum grating spectrographs. 1″ of air at atmospheric pressure, or ½ mm. of quartz, both transmit $\lambda = 1670$ A.

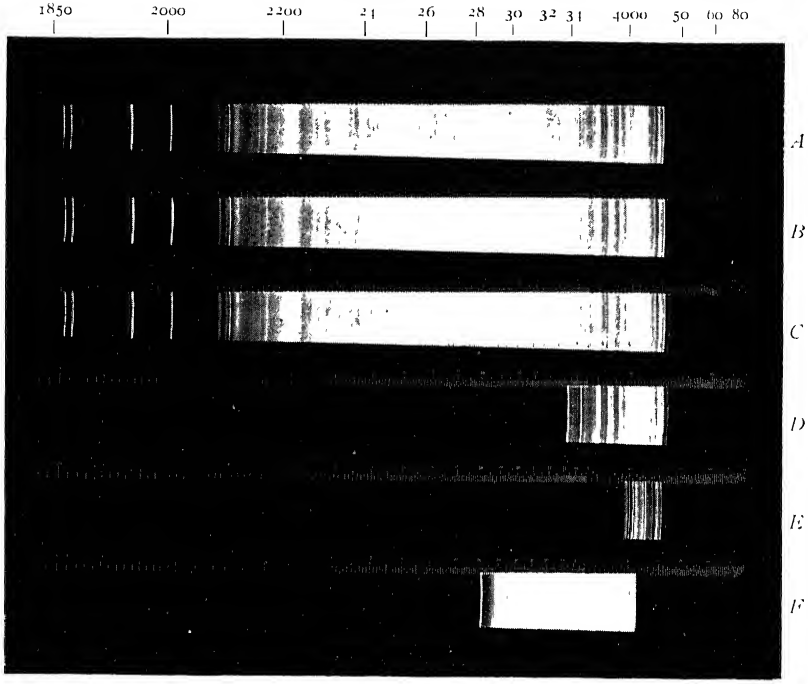


FIG. 1

A. Spectrum of Aluminium Spark (direct)
B Quartz
C Fused Silica
D Glass, Boro Silicate Crown, 18 mm.
E Glass, Dense Flint, 15 mm.
F Wood's Glass, 0·5 mm.

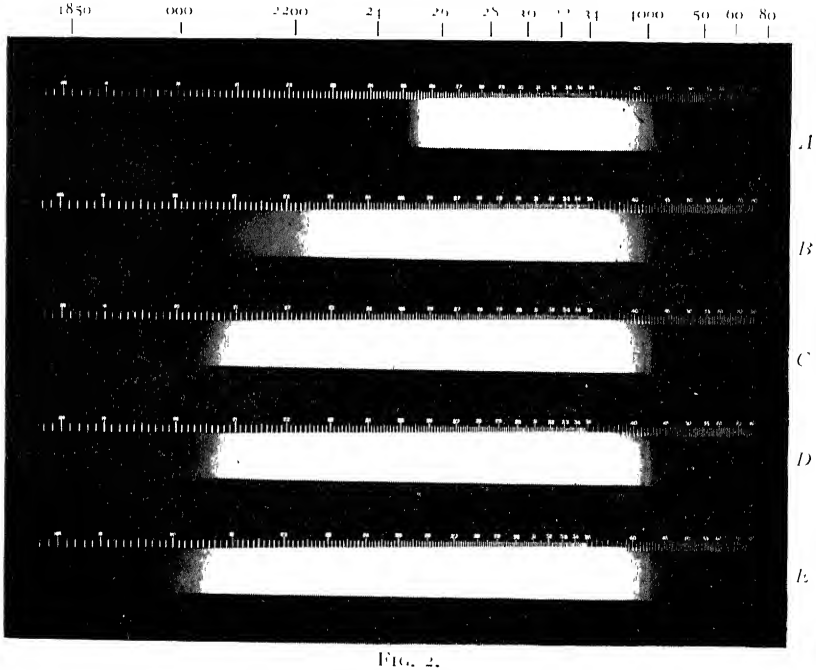


FIG. 2.

A. Carbon Tetrachloride, 1 mm
B Ethyl Alcohol, 1 mm
C. Cyclohexane, 1 mm
D Distilled Water, 1 mm
E. Spectrum of Hydrogen Discharge Tube (direct)

PLATE III

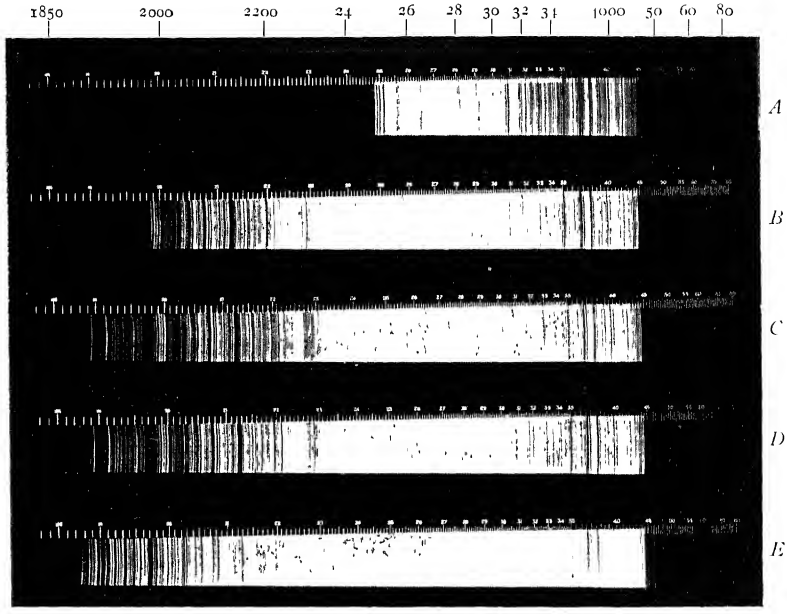


FIG. 3.

A. Carbon Tetrachloride, 1 mm
B. Ethyl Alcohol, 1 mm
C. Cyclohexane, 1 mm.
D. Distilled Water, 1 mm
E. Spectrum of Tungsten-steel spark (direct).

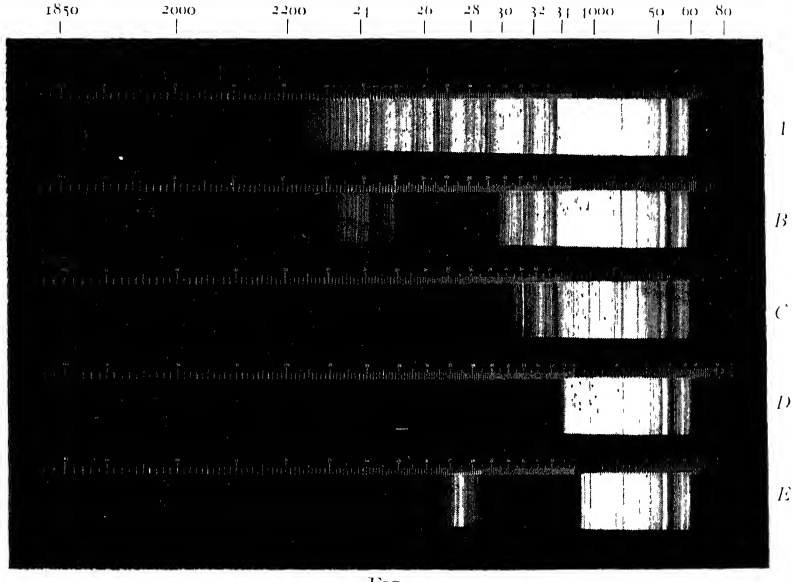
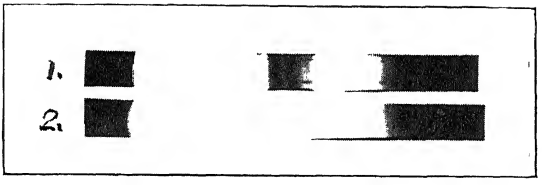


FIG. 4.

A. Spectrum of Iron Arc (direct).
B. 10% Solution of Acetone in Ethyl Alcohol. thickness 1 mm.
C. Canada Balsam thickness 0·1 mm
D. Monobromnapthalene thickness 2 mm.
E. Carbon Bisulphide thickness 2 mm.



ABSORPTION SPECTRUM OF ERGOSTEROL.
1. Before irradiation. 2. After irradiation.
Full size, photographed on Hilger E 37 Spectrograph.

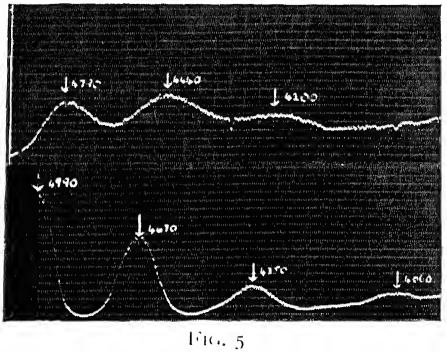


FIG. 5

ABSORPTION CURVES FOR β CAROTENE.
Above at ordinary temperature.
Below at liquid air temperature.
[*Reproduced by kind permission of Dr F. P. Bowden.*]

PLATE V

To face page 9

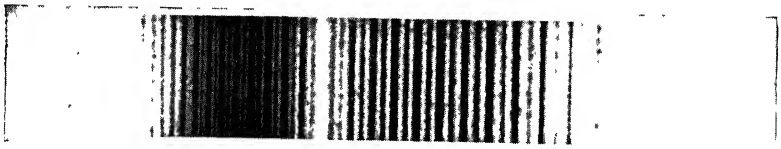


FIG. 6.

THE ABSORPTION BAND OF ACETYLENE AT 7887 A.

Photographed on an infra-red plate, using a grating ruled at the National Physical Laboratory (1 mm. on this illustration is of the order of 2 A). The dark represent absorption.

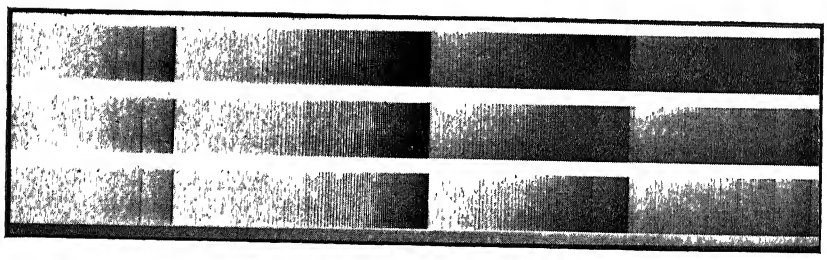


FIG. 7.

AN ELECTRONIC BAND SPECTRUM (N_2).

Photographed on a Hilger E Spectrograph. (Natural Size)

but the spectrum indicates that it is unsuitable for this purpose if observations at short wave-lengths are required. The application of monobromnaphthalene is limited in a similar way. The fifth and second exposures in this figure, however, which are of carbon bisulphide and of a 10% solution of acetone in alcohol (1 mm. tube) respectively, show examples of the other type, in which a region of absorption is followed by one of transparency, and then by further absorption.[1]

Finally, Fig. 5, which is reproduced by kind permission of Dr. F. P. Bowden, contains four spectra of biological interest. They are, respectively, those of ergosterol before irradiation, and of irradiated ergosterol, of β-carotene, and of β-carotene at very low temperatures. The last two spectra are reproduced, not in the form of the original photographs, but as the intensity curves obtained by examining these with a recording microphotometer, and their significance will be mentioned in Chapter III.

General and selective absorption.

It was formerly usual to distinguish between two kinds of absorption, general and selective. Where the intensity of absorption increases continuously towards one end of the spectrum or the other, the absorption was said to be "general", where the absorption varies in such a way that a graph of the percentage of light absorbed plotted against wave-length shows maxima and minima, it was said to be "selective." Thus, in Fig. 8, the part of the curve between A and B corresponds to general absorption, whilst in the wave-length range B-C the absorption is clearly selective.

In actual fact, there is no real distinction of the kind indicated by these words, as all absorption is selective, that is, is conditioned by the presence of absorption bands. Quartz, for instance, begins to absorb strongly in the infra-red at about 4μ. As this wave-length is approached the quartz begins very gradually to become less transparent, and the absorption increases with increasing wave-length in a way that would entitle it to be called "general". At 4μ the absorption rapidly becomes so intense that it produces almost complete opacity; but further into the infra-red, transmission begins again, and at 10·8μ a plate of quartz 2 mm. thick passes over 80% of the radiation which is incident on it. The regions of highly selective absorption between the two wave-lengths mentioned are also associated with the residual rays (*Reststrahlen*), that is, they include wave-lengths at which radiation is completely reflected, as if from a metallic mirror

[1] The absence of lines towards the red ends of these spectra is due to lack of lines in the aluminium spark and the other light sources spectrum, and to lack of sensitiveness in the photographic plates.

On the other hand, at very short wave-lengths, near and in the Schumann region of the spectrum ($\lambda < 2000$ A), the absorption of many compounds in both liquid and gaseous states often ceases to be purely selective, and appears to become "continuous," radiation of all wave-lengths now being completely absorbed. This phenomenon perhaps corresponds to decomposition of the molecules, whereas selective absorption seems to be more characteristic of definite groups, or arrangements of atoms, inside the molecules. Both selective and continuous absorption can thus be of great interest in chemistry.

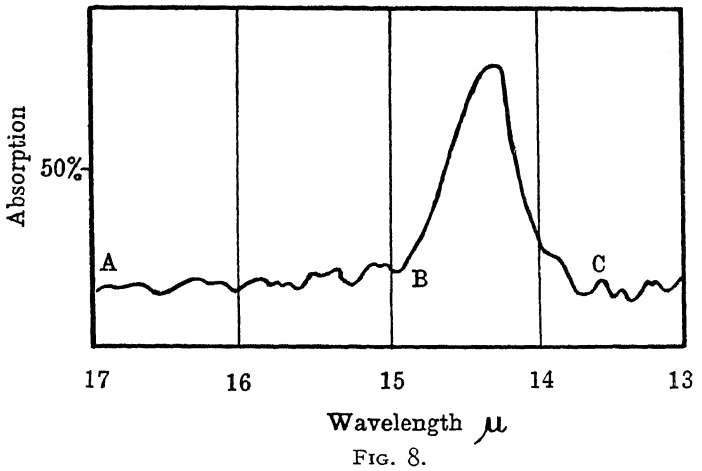


FIG. 8.

The Absorption of Witherite between 13μ and 17μ
(after Schaefer, Barmuth and Matossi)

The Quantum Explanation of the Emission and Absorption of Light.

Absorption of light has long been thought of as a resonance phenomenon. Probably it was Ångstrom who first conceived the notion that light is absorbed by a substance owing to the correspondence between the periods of vibration peculiar to the molecules and those of the light absorbed. On this hypothesis was formed his recognition of the correspondence between emission and absorption which in its clear statement by Kirchhoff in the well-known Kirchhoff's law[1] was the foundation of the science of astrophysics. Kirchhoff's law at once suggested that the dark lines in the solar spectrum were absorption lines due to vapours of substances which in terrestrial sources produce emission lines of the same wave-length.

[1] "Das Verhaltniss zwischen dem Emissionsvermogen und dem Absorptionsvermogen ist fur alle Korper bei derselben Temperatur dasselbe." Kayser, *Handbuch der Spectroscopie*, Vol. II. p. 16.

So long as the idea of absorption of light by resonance is not examined too closely it gives an easy and satisfactory picture, just as a few decades ago the idea of the atom with electrons rotating about a heavy nucleus was accepted as an explanation of the emission of spectrum lines and even as an explanation of the Zeeman effect. When, however, efforts were made to examine the explanation more closely, difficulties arose, although these did not entail the rejection of the basic conception.

Perhaps the most obvious difficulty in this pre-quantum explanation of the emitting atom is that the period of the rotating electron must, on classical theory, be that of the rotation in the orbit. But, on that same hypothesis, as the atom radiates energy it must necessarily change its orbit owing to loss of energy, with a consequent change of period, a deduction which makes the formation of sharp spectrum lines mysterious. It was to overcome this and other imperfections in the hypothesis that Bohr in 1912 applied the conceptions of the quantum theory to light emission. For this purpose, Bohr adopted the ideas of atomic structure which had been put forward by Rutherford on the basis of his fundamental experiments on the scattering of α-particles. On this theory, the atom is still pictured as consisting of a heavy nucleus, charged positively, around which is a collection of circulating electrons. In a normal atom, the number of electrons is equal to the nett positive charge of the nucleus. Sometimes, however, the atom loses one, two, or more of its electrons when it is said to be ionised. The charge of the nucleus determines alike the normal number of its cortège of electrons and the chemical nature of the element. Helium remains helium, in the sense that the nucleus can easily collect electrons and become a neutral atom of helium again, even when it has lost one or both electrons; and so with the other atoms. Such an atom may be regarded as capable of existing in a number of different states, to each of which pertains a definite energy of the system, but it is only when it changes from one of these states to another of less energy that it emits energy in the form of radiation.

In the course of the twenty years since it was first propounded, the original theory has naturally undergone considerable modification in its detailed application, but there appears to be no doubt that the process of radiation is a concomitant of a change of energy in the atom, that such changes can only take place by transition from one energy state to another, and that the character of the radiation is allied to the change of state of the atom in the following way:—For a given atom a series of energies E_1, E_2, E_3, etc., alone is possible. These energy states can persist without any radiation taking place, but if the energy of the atom is reduced from E_2 to E_1, the difference in energy $E_2 - E_1$ is emitted as a monochromatic radiation whose frequency ν of vibration is determined

by Bohr's relation $h\nu = E_1 - E_2$, h being the universal constant introduced by Planck in 1901, in his Quantum Theory of Radiation, which was based on considerations which at that time had no very obvious connection with spectral lines.

With the advent of wave-mechanics, the picture of the atom as a minute solar system is recognised as being too simple, but there remain as apparently permanent additions to knowledge the conceptions of energy levels in the atom, and of the relation between the wave-length of spectrum lines and these levels.

The necessary preliminary to emission is that an atom or molecule shall be raised to an energy state above the lowest, or ground state, in which the unexcited system exists. This may be done by raising its temperature in a flame or furnace, or by an electric discharge, or by the absorption of incident radiation. Considering, for simplicity, the case of the atom only, in the so-called arc spectrum, one of the outermost electrons may be shifted from its lowest energy state to any one of a series of higher states of energy. This process is only limited by the complete removal of the electron, when the atom is left in a " singly ionised " state. The return of the electron from the first higher state gives the first line of a spectral series, from the next higher state the next line, and so on. By bombarding the atom with electrons of suitably chosen and approximately uniform energy as exciting agent, it is possible to excite a series line by line, but in the arc all states are produced at once (in, of course, different atoms) owing to the lack of uniformity of exciting conditions, and all the lines of the series are observed simultaneously. The singly ionised atom is also capable, as an independent entity, of such step by step excitation, one of the remaining electrons acting as optical electron : the spectrum so produced is called the spark spectrum. Doubly and trebly ionised, and even more highly ionised atoms also have their own spectra, which lie further and further in the ultra-violet as the degree of ionisation is increased

But the transfer of the atom from the ground state to higher states can also be effected by the absorption of radiation. To every transfer corresponds the absorption of a quantum of radiation of appropriate frequency. Hence if radiation of a continuous range of frequencies be incident on the vapour of a monatomic element, *e g.* mercury, a series of absorption lines corresponding to transfers from the ground state will be observed, that is, the lines of the so-called principal series appear as absorption lines, and, in general, only these lines, since the light normally finds all the atoms in their ground state. Under certain conditions, however, some of the atoms may be found in excited states, and then absorption lines corresponding to these states as initial states will be observed.

The absorption spectra of molecules are much more complicated, and consist, not of lines, but of groups of bands, that is, spectral regions over which a whole range of wave-lengths is apparently absorbed. These are of three types, situated in the ultra-violet or visible spectrum, in the near infra-red, and in the far infra-red respectively.[1] The ultra-violet bands, like the lines produced by atoms, correspond to electron transitions. Those in the infra-red, however, have been attributed to oscillations relative to one another of the atomic nuclei composing the molecule in the case of the bands of shorter wave-length, and to rotations of the molecule as a whole in the case of those at very long wave-lengths. Under normal conditions, *i. e.* for liquids and solutions, and for vapours and gases at ordinary pressures, the bands have the appearance illustrated in Figs. 4 and 5, and the methods of absorption spectrophotometry described in Part II can easily be applied to them. In vapours and gases at lower pressures, however, the bands begin to show signs of discontinuity, and under suitable pressure conditions, examination with instruments of high resolving power has revealed in many cases that they consist of discrete lines, as in Figs. 6 and 7. These spectra may be assumed to be the true molecular spectra, the "fine structure" in which is blurred out by disturbing influences when the molecules are brought into close contiguity with other molecules, as they are in the solutions generally employed when the absorption spectrum of a liquid or solid substance is examined. From the point of view of molecular physics, the existence of fine structure inside the absorption bands is clearly of importance, and since it can be explained quite simply on the basis of the quantum theory, it has assumed fundamental significance.

The energy of a molecule is made up of three parts, that of its electrons, that associated with the vibrations of the atomic nuclei, and the energy of rotation. Each of these energies is quantised, just as the electronic energy of an individual atom is quantised. The molecule can thus exist only in a limited number of "electronic," "vibrational," and "rotational" states. The absorption bands in the far infra-red correspond to transitions from one such rotational state to another, and their long wave-length indicates that the energy changes involved are very small, about one-thousandth to one-hundredth of a volt.[2] The vibrational bands in the near infra-red, on the other hand, correspond to changes roughly 100 times as

[1] The "near" infra-red is the region of the spectrum extending from the red of the visible spectrum to a wave-length of about 23μ, whilst the "far" infra-red lies between this wave-length and 300μ, that of the shortest electric waves which can be excited by oscillatory electrical circuits.

[2] This is merely a conventional method of representing energies. A volt is, of course, not a unit of energy, but used in this sense, an "energy of 1 volt" is the energy which would be possessed by an electron after falling through a potential

large, of the order of 1/10 volt. A transition of the molecule from one vibrational state to another may be accompanied by a simultaneous rotational transition, which may be different from molecule to molecule, even though they are all undergoing the same vibrational change. The absorption spectrum which results, therefore, does not consist of a single line, but of a number of closely spaced lines separated by wave-lengths corresponding to the very small differences in the energies of the rotational states. It is these lines which constitute the fine-structure of the "vibration-rotation" bands, of which an example is shown in Fig. 6, reproduced by kind permission of Professor R. Mecke, of Heidelberg.

The fine-structure of the electronic bands (Fig. 7), although it is still more complicated, can be explained in a similar way. The energy required to produce spectra at wave-lengths in the middle ultra-violet region is of the order of 5 volts. Each electronic state or level in this region is associated with "sub-levels," about 1/10 volt apart (*i.e.* about 1/50 of the electronic energy) which correspond to the vibrational states in which molecules in one and the same electronic state may exist. The absorption resulting from a transition from one electronic state to another will therefore not consist of a single line, since different vibrational transitions may occur in different molecules simultaneously with the same electronic transition, but of a series of bands separated by about 1/50 of the wave-length of the electronic transition itself, *e.g.* by about 60 A at 3000 A. Each vibrational transition, however, as we have seen above, is also associated with various rotational transitions, so that each band will itself in turn have a fine structure with a separation between the lines some 100 times less, that is, about 1 A. Every electronic change occurring in a polyatomic gas or vapour thus produces a *band system* where an atom gives only a *single line*, and the whole series of such systems forms the complete electronic (ultra-violet) spectrum of the molecules. The total number of states of vibration and rotation of the molecule is quite large, and it might be expected that the electronic bands would contain a correspondingly large number of fine-structure lines. Fortunately, however, it seems that only certain rotational transitions are permitted, and

difference of one volt. Using the value of the electronic charge $e = 4{\cdot}77 \times 10^{-10}$ electrostatic units, or $1{\cdot}59 \times 10^{-20}$ electromagnetic units,

$$1 \text{ volt-electron} = 10^8 \times 1{\cdot}59 \times 10^{-20} = 1{\cdot}59 \times 10^{-12} \text{ ergs.}$$

The wave-length λ corresponding to an energy of V volts is easily deduced by means of the quantum relationship

$$Ve = h\nu,$$

h being Planck's constant, and ν the frequency. $\nu = c/\lambda$, where c is the velocity of light. $h = 6{\cdot}55 \times 10^{-27}$ erg. secs. $c = 3 \times 10^{10}$ cm. per sec

this limits the number, so that, at least for simple molecules, the spectra can still be analysed. In liquids, the intermolecular forces introduce serious disturbances, and the fine structure disappears, with the result that each electron transition produces a single wide continuous absorption band, of the kind which is normally observed with solutions of organic compounds.

Whatever the extent to which a complete physical theory of absorption may eventuate in the next few years, it is quite certain that measurements of absorption will become of increasing interest on theoretical grounds. But some of the chief applications of absorption spectra, *e.g.* in chemistry, are empirical in nature. Whether made for theoretical or for empirical purposes, such measurements to be of the fullest value must be quantitative, that is, an accurate determination of the proportion of energy absorbed at each wave-length must be made. Experimental methods specially designed for this end are described in Part II of this book, but before proceeding to a more detailed description of the instruments and their applications, it may be well to recapitulate the two principal laws of absorption, and to state the nomenclature now generally adopted in this field of research.

CHAPTER II

LAWS OF ABSORPTION AND THE NOMENCLATURE OF ABSORPTION SPECTROPHOTOMETRY.

Nomenclature and Notation.

The subject of absorption spectroscopy has been unfortunate in becoming encumbered with a too voluminous vocabulary. Many of the terms to be found in the literature are of little use or interest; many are redundant. As long ago as 1905, Kayser wrote: "It would be highly desirable that the uncertain use of various designations should be brought to an end. . . . In many papers the measurements are useless, as authors have neglected to describe clearly what they measure. For instance, if they give the 'absorption coefficient' one does not know what they understand under this name."[1] As a first attempt to introduce uniformity, Kayser

[1] Translated from *Handbuch der Spectroscopie*, Vol. III, p. 14 (1905).

proceeded to set out the nomenclature which he subsequently used throughout his "Handbuch," but unfortunately he himself used the term "extinction coefficient" in a sense different from that in which it was originally defined by Bunsen and Roscoe, as described below, which is the one generally adopted.

With the advent of modern methods of measuring absorption, the possibility of confusion increased and the necessity for the adoption of a standardised nomenclature became correspondingly more urgent. An attempt in this direction was made in 1924 in a preliminary draft of a report by Irwin G. Priest to the Committee on Standards and Nomenclature of the Optical Society of America. New terms were suggested, and these have been used in some of the publications of the Bureau of Standards. The draft was never completed or printed but particulars are to be found in Bureau of Standards Scientific Paper 440.[1]

The nomenclature there set out is different again from that employed by Victor Henri, who made the first *systematic* quantitative study of the absorption spectra of organic compounds.[2] The nomenclature used by Henri has been extensively used since by other workers, and has moreover been adopted recently in the International Critical Tables.[3] For this reason it will be used in the present book, and, for convenience of reference, it is reproduced in Table I.

Laws of Absorption.

Quantitative measurements of absorption are based on two fundamental laws concerning the relationship between the intensities of the light transmitted by a layer of an absorbing substance and that of the light incident on it.

1. *Lambert's Law* states that the proportion of light absorbed by a substance is independent of the intensity of the incident light. This law appears to be rigidly true in all experiments which have been made. The law can also be stated in the form that "each successive layer of the medium absorbs an equal fraction of the light passing through it". This statement is expressed in the equation

$$I = I_0 e^{-\mu l},$$

where I_0 and I are the intensities of the light entering and transmitted by a thickness l of the substance, and μ is a constant which is known as the

[1] p. 124 (1924). See also the report of the O S A. Progress Committee for 1922-23, "Spectrophotometry," *Journ. Opt Soc. Amer.*, Vol. 10, No. 2, p 169

[2] *Etudes de Photochimie* (1919).

[3] *International Critical Tables*, Vol. V, p. 359 (1929).

absorption coefficient of the substance.[1] The constant generally employed, however, is the *extinction coefficient*, which was defined by Bunsen and Roscoe as follows[2]:—"Let I_0 be the intensity of the incident light which enters the medium, I the intensity remaining after its subsequent passage through a path length l, and 1/K the path length, passage through which reduces the light intensity to 1/10th; then

$$I = I_0 . 10^{-Kl}$$

where K, a constant depending on the medium, is called by us the 'extinction coefficient.' l is to be taken in centimetres." The Bunsen-Roscoe extinction coefficient is, then, the reciprocal of the thickness which is necessary to weaken the light to 1/10th of its incident value. It is represented by K and it is related to the absorption coefficient μ by the equation

$$\mu = 2{\cdot}3026\ K.$$

2. *Beer's Law*, which was based on quantitative photometric observations on the absorption of red light by aqueous solutions of various inorganic salts,[3] states the relationship between the intensities of the incident and transmitted light in a different way, namely that the absorption is proportional to the number of molecules of absorbing substance through which the light passes. If the absorbing substance is dissolved in a non-absorbing medium, the absorption will then be proportional to the concentration of the solution. If I_0 is the intensity of the light entering the solution, I that after passing through a thickness l of it (expressed in centimetres), and c is the concentration, Beer's law can be expressed

$$I = I_0 . \alpha^{-cl}.$$

Here α is a constant for the absorbing solute and is obviously the ratio of the transmitted to the incident intensity for unit thickness and unit

[1] By "Intensity of Light" is meant the quantity of light energy incident on unit area in one second (ergs per cm^2 per sec), or in terms of quantum theory, the intensity of a given wave-length is the number of quanta of that wave-length or frequency reaching 1 cm.^{2} in 1 sec. In considering instruments for measuring absorption, account must be taken of the fact that although it can be arranged that the intensity is uniformly distributed in a given plane, if we limit that plane by a boundary (*e.g.* a slit or diaphragm) the intensity of the radiation will not be uniform at any other plane, unless this is an image of the first. Since I_0 and I refer to planes prior and subsequent to passage of the light through the medium, the phrase "intensity of light" cannot strictly have the same significance for both of them. If, however, for "intensity of light" we substitute "quantity of light energy" or "number of light quanta," this objection is removed.

[2] *Pogg. Ann.*, Vol. 177, p. 238.

[3] *Ibid.*, Vol. 86, p. 78 (1852).

concentration. If ε is written for K/c, the second expression for Lambert's law is converted into Beer's law in the form

$$I = I_0 . 10^{-lec}.$$

In other words the extinction coefficient, for a given wave-length, of a solution of concentration "c" is c times that of a solution of unit concentration, while for a given substance, cl is a constant for a given ratio of I to I_0. When c is expressed in gramme-molecules of absorbing substance per litre of solution, ε becomes a measure of the absorption due to a single molecule, and it is then known as the *molecular extinction coefficient.* It is this quantity which is most conveniently employed when comparing the absorption spectra of different substances.

TABLE I—NOMENCLATURE OF ABSORPTION SPECTROPHOTOMETRY

Term	Symbol	Definition
Density - - - - -	d	$d = \log_{10} I_0/I$
Extinction Coefficient - -	K	$I = I_0 . 10^{-lK}$
Molecular Extinction Coefficient	ε	$I = I_0 . 10^{-\varepsilon cl}$
Absorption Coefficient - -	μ	$I = I_0 . e^{-\mu l}$
Absorption Index - - -	k	$I = I_0 . e^{-4\pi kl/\lambda}$

I_0 = intensity of the light entering the medium.
I = intensity remaining after its passage through length l.
l = length of path in cms.
c = concentration of absorbing substance in solution expressed in gramme-molecules per litre.

Two other terms which appear in the literature of absorption spectrophotometry need to be mentioned here. The first of these is the *density* of a medium, introduced by Hurter and Driffield in connection with their researches on the blackening of photographic plates.[1] If I and I_0 have the same significance as in the previous paragraphs, then d, the density of the medium, is defined by the equation

$$I = I_0 . 10^{-d},$$

d is obviously related in a simple way to the molecular extinction coefficient since

$$d = \varepsilon cl.$$

For this reason, it is convenient to calibrate the scales of spectrophotometers to read directly in densities, and this is the general practice for the instruments constructed by Adam Hilger Limited which will be described

[1] *J.C.S.I.*, 9, May, 1890.

in Part II of this book. The term is useful because the total density of a number of media is equal to the sum of their separate densities.

The other term, known as the *absorption index*, k, is used mainly in mathematical papers on absorption. It is defined by the equation

$$I = I_0 \cdot e^{-4\pi kl/\lambda},$$

where λ is the wave-length of the light for which the absorption is to be specified. k can be converted into ε by the simple relationship.

$$k = 0{\cdot}183\ c\lambda\varepsilon.$$

The Validity of Beer's Law.

Beer's law was tested as long ago as 1873 by Vierordt, who devised the first method of absorption spectrophotometry with any claim to accuracy,[1] and it was repeatedly confirmed by other workers after him. Nevertheless, despite the lack of sensitivity of the semi-quantitative methods which were generally employed before the development of modern spectrophotometers, apparent exceptions were reported from time to time, so that in 1917 the statement was made that " this ' law ' of Beer has been shown to be the exception rather than the rule." [2] Since then, a number of very accurate investigations of the validity of both the laws of absorption have been carried out. Prominent among these is the work of von Halban,[3] who, after reviewing all the measurements made in the preceding twenty years, concluded that deviations from Beer's law can generally be attributed to chemical change in the absorbing molecules. Measurements by the very sensitive photo-electric method which he had developed showed that the law is actually obeyed in very dilute solutions of substances which had previously been reported to behave abnormally, His observations on solutions of potassium chromate also confirmed Lambert's law very exactly.

The deviations from normal behaviour exhibited by cobalt salts, which had led Hulbert to reject Beer's law, had already been attributed to the formation of complexes More recent examples in which such deviations can be explained on chemical grounds are the variation with dilution of the absorption spectra of iodine in various solvents, attributed to the presence of I_6 molecules,[4] and similar variations in the case of tartaric acid, which can be explained in exactly the same way as the anomalous rotatory dispersion of this compound, namely that the solutions contain

[1] K. Vierordt, *Die Anwendung des Spektralapparats zur Photometrie der Absorptionsspektren und zur quantitativen chemischen Analyse.* Tübingen, 1873.

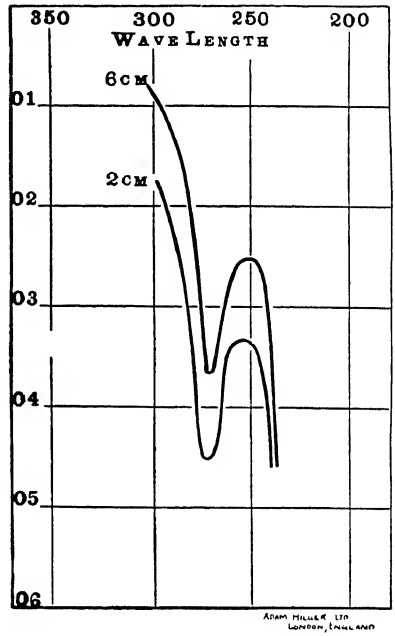
[2] Hulbert, *J. Phys Chem.*, Vol. 21, p. 150 (1917).

[3] See Halban and Ebert, *Zeits. phys. Chem*, Vol. 112, p. 321 (1924).

[4] Groh and Papp, *Zeits. phys. Chem.*, A, Vol. 149, p. 153 (1930).

two forms of the acid molecule in equilibrium.[1] In many cases, *e.g.* for most derivatives of benzene and those of many heterocyclic compounds,[2] for various electrolytes at high dilutions, and even for colloidal solutions,[3] the law has been shown to be strictly valid.

It seems safe to conclude, therefore, that when the absorption of a substance does not follow Beer's law, the reason is to be sought, not in



5 592m $CaCl_2$ in 2 cm tube 1 864m $CaCl_2$ in 6 cm tube
Ordinates represent molecular extinction coefficients.

FIG. 9.

invalidity of the law, but in some change in the absorbing molecules, such as the formation or disintegration of molecular aggregates, interaction between solvent and solute, or disturbance of equilibrium, depending on concentration, between two types of absorbing molecule present in the system. A discussion of some of the problems involved here is to be found in a recent paper by Darmois.[4]

Fig. 9 is a typical example of the kind of result obtained when Beer's law is not obeyed. For solutions in which it is strictly valid, the law can

[1] Lucas and Schwob, *J. de Phys.*, Vol 3, p. 53 (1932).

[2] Goslawski and Marschlewski, *Acad. Polonaise Sci et Lettres, Bull.*, 4-5, A, p. 383 (1931).

[3] Lange, *Zeits. phys. Chem.*, A, Vol. 159, p. 277 (1932).

[4] Darmois, *Comptes Rendus*, Vol. 197, p. 1120 (1933).

be applied directly to the analysis of mixtures of two substances, provided that their absorption spectra are sufficiently different from one another. The algebraic expression of the analysis for two such cases is set out on pages 41-44, and applications will be mentioned in the next chapter.

CHAPTER III

SOME RESULTS AND APPLICATIONS OF ABSORPTION SPECTROPHOTOMETRY AND THE RAMAN EFFECT

Earlier Work.

The earliest recorded observations of absorption spectra were made by Brewster in 1833, though the first serious work done in the ultra-violet appears to have been that of W. A. Miller and G. G. Stokes. In 1862 Stokes and Miller independently communicated to the Royal Society the results of experiments on the transparency of various substances in the ultra-violet.[1] In Stokes' instrument the ultra-violet rays were rendered visible by fluorescence, but Miller's was a true quartz spectrograph. With this instrument he determined the absorption of a number of substances. Of all the substances he tried, ice (and water), fluorspar and hydrate of ammonia were the only ones which did not cut off some of the rays transmitted by the quartz spectrograph—although some other liquids examined by him (alcohol, glycerine) must, one supposes, have been impure, since had they not been so he would have found them no less transparent than water. He also tried gases, and the reflection from polished surfaces of many kinds—finding instances of absorption in both sets of experiments. In this paper he describes the selective absorption of silver,[2] and the absorption spectra of 21 solids (eight of them being glasses), 109 solutions and other liquids, 25 gases and 16 polished reflecting surfaces.

In 1872, Hartley came into possession of the instrument which Miller had used, improved it and continued the earlier investigations.[3] Miller had been unable to find any relation between constitution and absorption spectra, although he writes, "The most interesting fact, however, disclosed by these various experiments is the persistence of either the diactinic or the absorbent property in the compound whatever be its

[1] *Phil Trans*, 152 II. [2] *Ibid.* p. 875.

[3] *Sci Proc Roy. Dublin Soc* (3) 3, p 93 (1883).

physical state—a circumstance which proves that the property under examination is intimately connected with the atomic or molecular nature of the body, and not merely with its state of aggregation."[1] We shall see that Hartley was to have somewhat better success.

During the hundred years which have elapsed since Brewster's observations, the spectra of an enormous number of substances have been measured. So significant is the information which can often be obtained in this way that the absorption spectrum is now one of the first properties of a newly produced compound to be investigated. Unfortunately, much of the work done prior to the last twenty years was valueless, since the methods employed were either entirely qualitative, or at best, semi-quantitative, compared with those available to-day. Some uniformity was introduced, however, by Hartley and other workers after him who developed a method in which the wave-lengths at which various thicknesses of a given solution ceased to transmit were recorded photographically. By diluting the solution progressively, the course of the absorption could be followed up to its maximum of intensity, and a curve of equivalent thicknesses of standard solution (or the logarithms of these thicknesses) as ordinates against the observed limiting wave-lengths transmitted as abscissae then reproduced the main characteristics of the absorption band.[2] Whilst the wave-lengths of the maxima of absorption so recorded are fairly trustworthy, these curves do not give any accurate idea of their intensity, nor, unless the conditions of measurement were identical, can the curves for two different substances be compared with one another. The method also fails to reveal the detailed course of the absorption, as can be seen by comparing the absorption spectrum of benzene as recorded by Hartley, Fig. 10, (reproduced from Kayser[3]), with the curve shown in Fig. 25, which was obtained by a modern photometric method.

On the basis of extensive measurements of absorption spectra of this kind Hartley, and a good deal later Baly and others, attempted to establish a relationship between the chemical constitution of an organic molecule and its absorption spectrum. They found that similarity in absorption spectrum does in fact often correspond to similarity in structure, and they were able to apply this result to the elucidation of problems of molecular structure when ordinary chemical methods had failed. It was found possible to attribute the development of colour in a compound to the presence of certain " chromophoric " groupings which were known to be present in dye-stuffs, whilst absorption measurements were also

[1] *Journ. Chem. Soc.*, vol 17, p. 75 (1864)

[2] A description of the method can be found in Kayser's *Handbuch der Spectroscopie*, Vol. III, Chapter 3 of which was written by Hartley ; or in Baly's *Spectroscopy*.

[3] *Handbuch d. Spectroscopie*, Vol. III, p. 175.

applied to the study of tautomerism, as in keto-enol equilibria.[1] More modern work, however, has shown that applications of this type may often lead to false conclusions, and should only be made with great caution. Further reference to this will be made below.

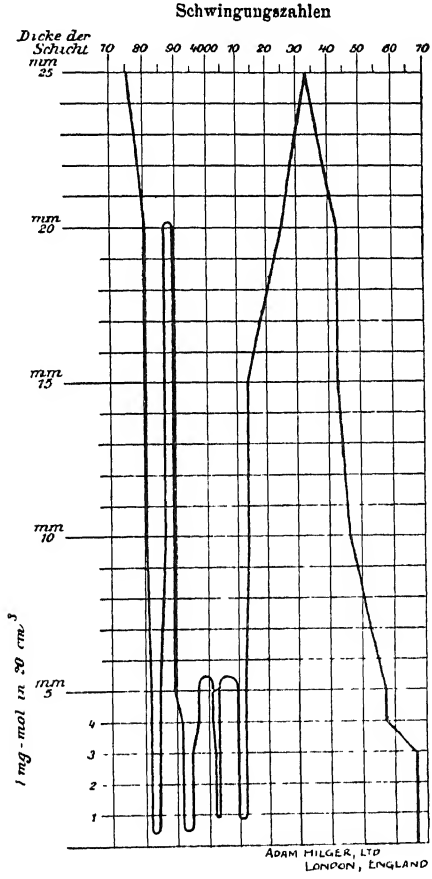


FIG. 10.

THE ABSORPTION SPECTRUM OF BENZENE (HARTLEY) REPRODUCED FROM KAYSER'S *Handbuch der Spectroscopie.*

The Investigations of Victor Henri.

One of the disadvantages of these earlier measurements was that they were made chiefly with substances of very complex structure. The

[1] For an account of this earlier work, see Baly's *Spectroscopy*, or Smiles, *The Relationship between Chemical Constitution and some Physical Properties*, or Kayser *loc cit* Hartley must undoubtedly be considered the pioneer in this branch of the subject.

first systematic investigation of the ultra-violet absorption spectra of simple organic compounds was carried out by Victor Henri and his pupils.[1] Henri employed a quantitative method which enabled him to plot molecular extinction coefficients ε against wave-lengths λ. It depends on the result obtained by Schwarzschild that the blackening of a photographic plate is related to the intensity I of the light incident on it and the time t of exposure by the expression

$$\text{density of blackening} \propto It^n,$$

where n is a constant depending only on the type of plate and the method of development. Spectra are photographed alternately through equal lengths l of solvent, for time t_0, and solution, for time t, and the wave-lengths at which the blackening produced on the plate is the same in each spectrum are obtained. If I_0 and I are the respective transmitted intensities, then at these wave-lengths

$$I_0 t_0^n = It^n.$$

It is clear that I is virtually that part of an incident intensity I_0 which would be transmitted by the solute alone, so that

$$d = \log_{10} I_0/I = n \log_{10}(t/t_0).$$

The method involves an independent determination of n, but it is a great improvement on the earlier " equivalent thickness " method, although of course, it is not capable of the high degree of accuracy now obtainable by photometric processes. Nevertheless, the results obtained were of great value, and the method is still often used for special purposes when others are not so conveniently applicable.

The first result of Henri's investigations was to show that the absorption spectrum of a compound is not specifically characteristic of the whole molecule, but only of particular radicals in it For example, all simple ketones exhibit an absorption band, with a maximum intensity about $\varepsilon_{max} = 15$, between 2700 and 3000 A. The absorption curve has the same characteristic shape for all ketones, but in a homologous series, the maximum moves towards longer wave-lengths, and its intensity increases as the weight of the molecule increases Other radicals behave in a similar way, and Henri extended the use of the term " chromophor," which had previously been applied to the particular groups which could be associated with visible colour, to cover any radical which gives rise to such a characteristic absorption spectrum.

When more than one chromophoric group is present in the molecule, the appearance of the absorption spectrum is changed, but Henri was able to identify two types of effect, and while later work has shown that this

[1] A résumé of this work is contained in *Etudes de Photochimie* (1919).

classification is by no means so rigid as he at first suggested, it nevertheless provides a useful guide in the analysis of more complicated spectra. Thus, when the chromophoric groups are separated in the molecule by two or more saturated C – C linkages, the spectrum contains the bands characteristic of each, perhaps enhanced in intensity. If the chromophors are closer, and, in particular, if they form part of a "conjugated" configuration, the bands may either be displaced considerably towards longer wave-lengths, or a completely new absorption system, now characteristic of the conjugated structure, may be produced. An example of the former type is furnished by camphor, exhibiting the single band corresponding to its single $>C=O$ group, and camphorquinone, with two such groups, which absorbs in the visible spectrum and is coloured yellow. The conjugation of two ethylenic groups, on the other hand, appears to produce a completely new absorption band at longer wave-lengths than, and quite different in character from, that of a single C = C bond. This displacement of the absorption towards longer wave-lengths often gives rise to colour, as in the multiply-conjugated compound carotene and in other similar natural plant colouring matters.

The Identification of Unknown Substances.

On the basis of such generalisations, tentative though they are, it is possible to begin to apply the ultra-violet absorption spectra of liquids or solutions to the elucidation of problems of molecular structure, without recourse to methods depending on observations of *fine-structure* in infra-red or ultra-violet bands of gases and vapours, which will be described below. They must always be used, however, with great caution, and only in conjunction with other methods of approach. A *similarity* which does not amount to an *identity* of spectra can only be interpreted as indicating *similarity* of structure. In the same way, analysis based on observations of absorption alone may easily lead to false conclusions. This is particularly the case in biological chemistry, where the substances concerned are usually complex, in which case small differences in constitution do not produce such radical changes in the absorption spectra. Thus, serum pseudo- and eu-globulin have similar absorptions.

However, given that the absorption spectrum of the unknown substance is similar to that of a known one, identity can be established if the necessary additional information is available. This can be obtained in various ways: (*a*) from similarity of chemical behaviour or analysis; (*b*) from other physico-chemical properties, *e.g.* optical rotatory power if the substance is optically active; (*c*) from similarity in physiological activity, as in drugs or the vitamins; or (*d*) from a study of the behaviour of the absorption under different conditions, *e.g.* at very low temperatures, as

will be described below. An example of the application of such factors was the identification of very minute quantities of the unknown substance vitamin A in certain oils, by Woodrow,[1] and by Morton and Heilbron.[2] A similar observation was made by Capper,[3] who found that substances extracted from the liver of rats fed on carotene gave absorption bands identical with those of vitamin A. Warburg [4] has also been able to establish the identity and function of the respiratory ferment by correlating the absorption spectrum of the living cells with the variation with wave-length of the photochemical effectiveness of the incident light.

Quantitative Applications of Absorption Spectra.

Once the absorption spectrum of a compound has been established and it is known that the solutions obey Beer's law, this law may be applied to quantitative analysis. Experimentally, the problem is one of determining the extinction coefficients with great precision for a few, or over a narrow range of wave-lengths, and the method employed must be adapted for this purpose. An important application of this type has been in the estimation of alkaloids, which, according to Eisenbrand,[5] can be detected in quantities of 0·1 mg by absorption methods. The method also has its use in the control of the purity of foodstuffs, and these two special subjects are described at greater length in an Appendix at the end of Part II. The recent rapid developments in the study of the vitamins, and their preparation on a commercial basis, have demanded constant applications of absorption spectrophotometry, both for purposes of identification and for quantitative estimation of the products. In the case of vitamin A, the process has now been reduced to a matter of routine practice by the introduction, by Adam Hilger Limited, of a new specialised instrument, the Hilger Vitameter A, for measuring the vitamin A chromogen content of fish liver oils, their extracts and other substances [6] The absorption method has been employed in the quantitative analysis of nitrates in the presence of nitrites and other anions [7] provided that no absorbing cation is present, whilst it has proved of great value in the determination of terpenes in their industrial preparation,[8] very small

[1] *Phil. Mag.*, p. 944, 1928.

[2] *Biochem. Journ*, XXI, 1, p. 78, 1927; XXII, 4, p. 987, 1928.

[3] *Biochem Journ*, XXIV, 4, p. 980, 1930.

[4] See *Physiological Reviews*, Vol. X, 1930.

[5] *Pharmaz. Zeitung*, Vol. 71, p. 716 (1926).

[6] A further instrument, the Vitameter A.C., will also estimate the Vitamin C (ascorbic acid) content of fruit juices etc.

[7] *Pharmaz. Zeitung*, Vol. 72, p. 672 (1927).

[8] Muller, *Riechstoffindustrie*, p. 98, 1927.

amounts of impurity producing a measurable effect. Other purification processes in which absorption spectra have been employed are the preparation of pure phenanthrene, fluorene, and anthracene,[1] and in rubber refining.[2]

The determination of molecular extinction coefficients with great accuracy over a range of several hundred angstrom units in wave-length has recently become of great importance in connection with a physical theory of optical rotatory power, in which the total rotatory power of an optically active compound is resolved into " partial rotations " contributed by certain of the absorption bands. The partial rotation from a band can be calculated if the intensity of the band, its " half-width " (*i.e.* the difference between the wave-lengths at which $\varepsilon = \frac{1}{2}\varepsilon_{max}$), and the corresponding circular dichroism (difference in molecular extinction coefficients for light which is circularly polarised in left- and right-handed senses respectively) are known. The mathematical analysis involves a knowledge of the relationship between the molecular extinction coefficient ε and the frequency ν, which is not yet conclusively established, and for which measurements of high precision are required in order that valid empirical relationship may be found.[3]

Such measurements have also found an application in biological problems. Thus, absorption curves have been established for purified proteins in order that observations of absorption spectra may be used for the identification and estimation of immunological precipitates. If the nitrogen content of the protein is known and the extinction coefficient is that of the pure protein for the same concentration of nitrogen, then it can be fairly assumed that one is dealing with the same protein.

The first spectrophotometric measurements of the serum proteins were made by Judd Lewis[4] who found a marked difference between horse and human proteins. These were repeated but not confirmed by Campbell Smith[5] who showed that horse and human serum albumin and globulin give respectively the same absorption curves. The absorption of pseudo-globulin is about double that of albumin and occurs at the same wave-length (Fig. 11). A large number of experiments since made by Campbell Smith on pseudo-globulin obtained from rabbit, ox and goat and purified in several different ways have confirmed his original results. By making use of the extinction coefficient obtained for pseudo-

[1] Capper and Marth, *Journ. Chem. Soc*, p. 724 (1926).

[2] Scheibe and Pummerer, *Berichte*, Vol. 60, p. 2163 (1927).

[3] See Lowry and Hudson, *Roy. Soc. Phil. Trans.*, A, Vol. 232, p. 117 (1933).

[4] *Proc. Roy. Soc*, B, p. 178 (July) 1922.

[5] *Proc Roy. Soc.*, B, Vol. 104, (1929).

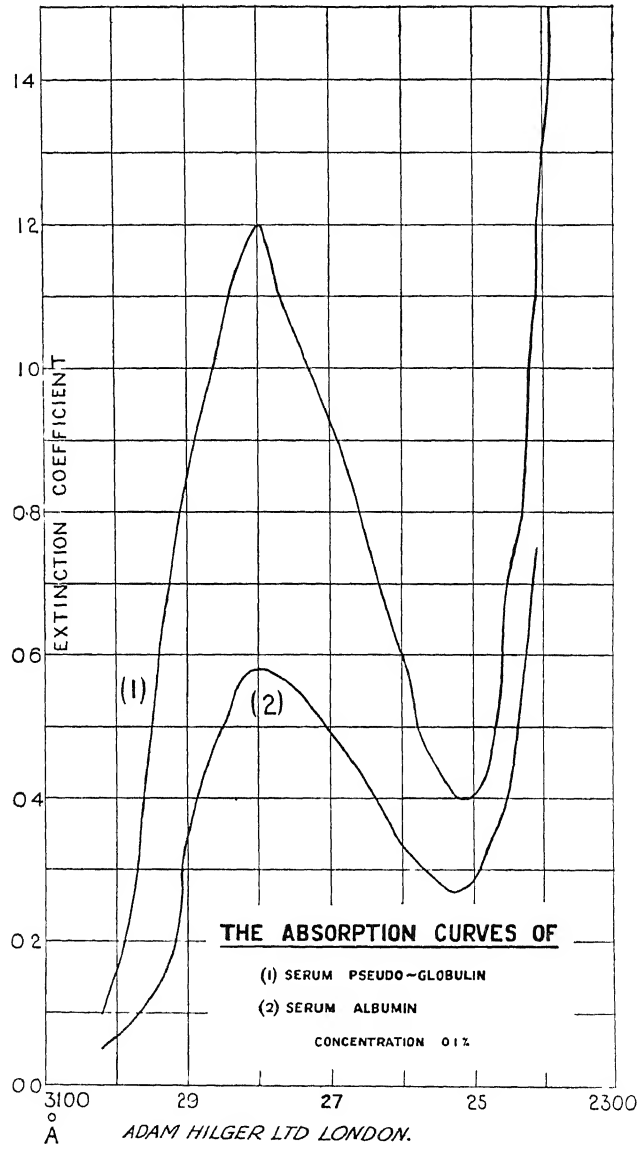


FIG. 11.

globulin, Marrack and Campbell Smith[1] have investigated the nature of diphtheria toxin antitoxin floccules (the so-called Ramon precipitate). They have shown that the absorption spectrum of the floccules formed by

[1] *Proc. Roy Soc.*, B, Vol 106, (1930).

a balanced mixture of diphtheria toxin and antitoxin are identical with that of serum pseudo-globulin. Further, they have shown that the reaction is a quantitative one and apparently independent of the conditions under which flocculation takes place, particularly the presence of non-specific protein. The results seem to show that such an immunity reaction is of the nature of a specific chemical reaction and indicates a new approach to problems of this kind. It should be noted that the method is dealing with quantities of only 2 or 3 mgs. of protein, the concentration of which can be measured to within 3 or 4%.

Another application of this method is to the estimation of purines. It has been known since the work of Soret[1] and Dhéré[2] that all purines show selective absorption in the ultra-violet between the range 2950 A-2500 A. Their extinction coefficients are very high so that they can be estimated in extremely dilute solution ; a solution of uric acid containing 2·6 mgm. in 100 c.c. can be estimated with an error of about 3% and about a tenth of that concentration could be detected in an otherwise optically transparent solution. The ability to measure such small amounts has been stressed as it seems likely that the spectrophotometer could be made to form the basis of a microanalytical method for purine estimation.

Campbell Smith[3] showed that a protein-free filtrate of blood serum gave an absorption curve very similar to that of a simple solution of uric acid. The amount of uric acid needed to give this absorption was extremely close to the amount found by chemical determination of uric acid in the plasma. Holiday[4] measured the absorption curves of some purines and showed that a purine combined with a molecule having no appreciable absorption at the same wave-length, as in a nucleotide or nucleoside, showed the same absorption as the simple purine. He has made use of this to verify the presence of combined uric acid (which is not determined by the chemical methods) in ox blood It is a noteworthy point in connection with the above work that the method is able to estimate the presence of a substance—namely, combined uric acid—which the chemical method is unable to do without first submitting the chemical complex to a tedious hydrolysis.

A similar, but slightly different application of absorption spectrophotometry is the study of equilibria existing in chemical systems For this purpose, direct chemical methods break down in that they almost

[1] *Arch. Sci Phys Nat*, 3e periode, p. 429 (1883).

[2] *Recherches Spectrographiques sur l'Absorption des Rayons Ultra-violets par les Albuminoides, les Protéides, et leurs Derivés*, Fribourg (1909).

[3] *Biochem Journ*, XXII, 6, p 1499 (1928).

[4] *Biochem Journ*, XXIV, 3, p. 619 (1930).

inevitably involve a disturbance of the system which is being examined, and the absorption method thus has an immediate advantage. The difference from ordinary quantitative estimation is that in this case it is generally small changes in intensity of absorption which require to be measured, although, of course, an absolute value may have to be determined as well. The earlier work on keto-enol tautomerism has already

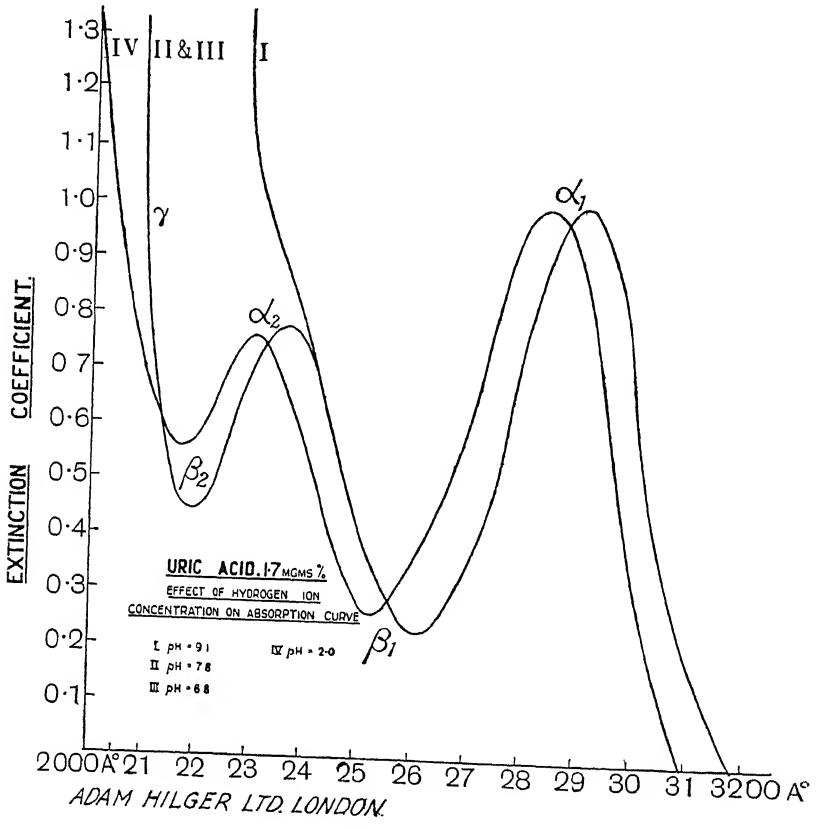


FIG. 12.

THE INFLUENCE OF *p*H ON THE ABSORPTION SPECTRUM OF URIC ACID.

been mentioned, and more recent examples are the determination of the dissociation constant of violuric acid [1] and of phenol and the hydroxyl group of tyrosine.[2] The association of formic acid [3] and equilibrium

[1] Morton and Tipping, *Journ. Chem. Soc.*, p. 2514 (1925); p. 1398 (1927).

[2] Stenstrom, *Journ. Phys. Chem.*, Vol. 29, p. 1477 (1925); Vol. 30, p. 1683 (1926).

[3] Ramsperger and Porter, *Journ. Am. Chem. Soc.*, Vol. 48, p. 1267.

constants of molecular compounds such as the alkali trihalides [1] have also been investigated in this way, whilst Halban has applied his very sensitive photoelectric method to a study of the equilibria between aromatic nitro-compounds and hydrocarbons at various temperatures, and has been able to deduce the heat tone of the reactions from the temperature coefficients so determined.[2]

Further interesting applications of this type can again be found in biological chemistry. Thus, hydrogen ion concentration is known to influence the absorption spectra of some biological substances, the bands moving towards shorter wave-length with increasing pH, whilst a change in the shape of the curve may also occur. Campbell Smith [3] showed that uric acid exhibits two bands in acid solution and only one in alkaline solution. In Fig. 12, the change of this spectrum with pH is reproduced.

Molecular Spectra.

In Chapter I, three types of molecular band spectra were described, namely, the rotation spectrum of the far infra-red, the vibration-rotation spectrum of the near infra-red, and the electronic spectrum in the ultra-violet, and a brief account of the origin of the fine-structure lines which make up these bands was given. Interesting and important as are the results and applications of the continuous bands exhibited by liquids and solutions which have been outlined above, the results of analysis of this fine-structure in the spectra of the vapours, although the work is still in its infancy and is confined almost entirely to molecules of simple structure, are likely to prove far more fundamental. To quote from an article by Professor Mecke, of Heidelberg, who has been one of the pioneers in this field, " the spectroscopist works on the ' living ' molecule. His method is that of stethoscopy and percussion. The dimensions of the molecule are measured directly—not indirectly—and with the spectroscope as stethoscope he ' sees,' in the truest sense of the word, the heart beat of the molecule, and from its frequency he can draw conclusions as to the constitution and power of resistance of the molecule." [4]

The total energy E of a molecule is made up of three parts,

$$E = E_{\text{electrons}} + E_{\text{vibration}} + E_{\text{rotation}},$$

of which the electronic is by far the largest and the rotational by far the

[1] Job, *Comptes Rendus*, Vol. 182, p. 632 and p. 1621.

[2] *Zeits. phys. Chem*, Vol 117, p. 461, (1925)

[3] *Biochem. Journ.*, XXII, 6, p. 1499 (1928).

[4] Translated from Mecke's article " Bandenspektren und Stereochemie " in Freudenberg's *Stereochemie* (1932).

smallest. If, when the molecule absorbs radiation, only the rotational energy changes (*rotation spectrum* in the far infra-red), the quantum absorbed

$$h\nu = \delta E_{rot}.$$

It can be shown that

$$E_{rot} = h^2 m(m+1)/8\pi^2 I,$$

h being Planck's constant, I the moment of inertia of the molecule about the axis of rotation, and m the rotational quantum number. In this expression, m is the only variable, and the quantum theory shows that it may only change by a single unit, i.e. $\delta m = +1, 0$, or -1. The result is thus obtained that the lines making up the rotational spectrum will have frequencies given by

$$h\nu_{rot} = h^2\,[(m+1)m - m(m-1)]/8\pi^2 I = 2mh^2/8\pi^2 I, \text{ or } \nu_{rot} = 2mh/8\pi^2 I.$$

Since m can have values 1, 2, 3, ... etc., the lines will be separated by constant frequency intervals $2h/8\pi^2 I$. It is customary to express such intervals, not in actual frequencies, but in *wave-numbers*,[1] and the result is then written in the form 2B, where $B = h/8\pi^2 cI = 27{\cdot}7 \times 10^{-40}/I$, ($c$ being the velocity of light $= 3 \times 10^{10}$ cm/sec.), so that the moment of inertia I, and thence the dimensions, of the molecule can be deduced directly from observations of this separation. Unfortunately, such observations in the far infra-red are very difficult to carry out, and in addition, molecules which do not possess a permanent electric moment do not exhibit rotational spectra, so that data from this source are available for only one or two compounds (*e.g.* H_2O, NH_3, and the hydrogen halides). For non-polar molecules, the same information can be obtained from the Raman effect, which is described later. It is interesting that in actual practice the separation of the rotational lines is not constant, but falls off a little with decreasing wave-length, as the molecule becomes distorted by its rotational motion and the moment of inertia becomes larger.

When both rotational and vibrational energies change simultaneously (*vibration-rotation* spectra of the near infra-red),

$$h\nu = \delta(E_{rot} + E_{vib}).$$

This spectrum lies at more accessible wave-lengths, and can easily be examined, for instance, by means of the Hilger Infra-red Spectrometer, and in some cases can be photographed using a diffraction grating and infra-red sensitive plates.[2] Such a photograph is reproduced in Fig. 6.

[1] Wave-number $= 1/\lambda = \nu/c =$ true frequency/velocity of light. See p. 6.

[2] For photographic plates for the infra-red, see p 113.

Many more data are thus available than for the simple rotation spectrum. The quantum theory leads to the result that the vibrational energy of the molecule is

$$E_{vib} = (n + \tfrac{1}{2})h\omega_0,$$

where n is the vibrational quantum number ($n = 0, 1, 2$, etc.) and ω_0 is the fundamental vibration frequency. Adding the rotational energy

$$E_{vib} + E_{rot} = (n + \tfrac{1}{2})h\omega_0 + h^2m(m+1)/8\pi^2I.$$

Thus for a simultaneous transition from the vibrational level n_1 to n_2 and the rotational transition m_1 to m_2

$$\delta E = h\omega_0(n_2 - n_1) + h^2[m_2(m_2+1) - m_1(m_1+1)]/8\pi^2I.$$

But, as before $(m_2 - m_1)$ is generally restricted to the value 1, and for the "*fundamental*" vibration band, $n_2 - n_1 = 1$, so that we can write

$$\delta E = h\omega_0 + h^2m/8\pi^2I,$$

or, for the frequency absorbed,

$$\nu = \omega_0 + hm/8\pi^2I.$$

The first term in this expression fixes the position of the centre of the vibration band, whilst the second term represents the rotational fine-

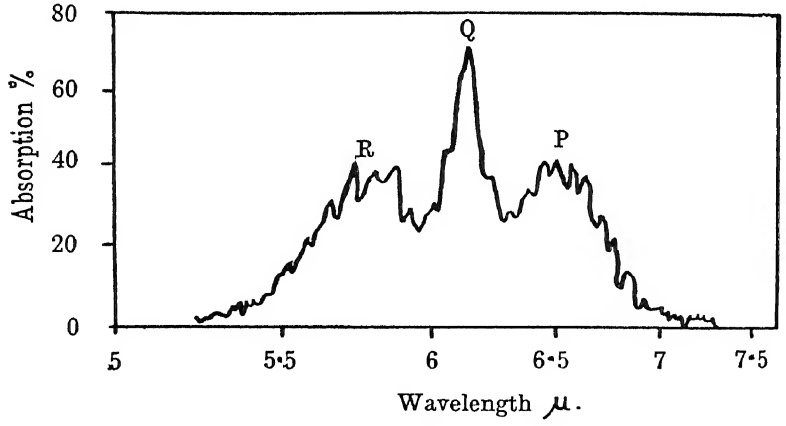


FIG. 13.

Absorption of Ammonia at 6·13μ
(after Robertson and Fox, *Proc. Roy. Soc.*, A, 120, 169).

structure separation (as m varies unit by unit) in it. The moment of inertia I can then be deduced from observations of this separation in

bands which lie in a region of the spectrum which is much more accessible than that of the pure rotation spectra.

One interesting feature of the rotation-vibration spectra is that for a diatomic molecule whose moment of inertia about a line joining the nuclei is zero, m may have values ± 1, ± 2, etc., but never o. When m is positive, a series of lines lying on the higher frequency side of ω_0 is obtained. These lines form what is known as the " R-branch " of the band. Similarly, for the negative values of m, a " P-branch " at lower frequencies is formed. In molecules where the value $m = o$ is permitted, a group of lines about the fundamental frequency ω_0 is also obtained, but the presence of such a " Q-branch " always indicates the possibility of the molecule possessing angular momentum about an axis joining the nuclei. Q-branches are frequently observed with polyatomic molecules, but rarely in diatomic molecules, NO being the only case on record. Fig. 13 shows the P, Q and R branches of the absorption-band of ammonia at 6·13 μ.

The information which can be derived from a study of vibration-rotation spectra is (1) the moment of inertia of the molecule about an axis of rotation, giving the molecular dimensions, and (2) the "restoring force " of the vibrations which is deduced simply from the fundamental frequency ω_0 and the mass of the molecule, and which gives a measure of the strength of the chemical bond.

The analysis of the electronic bands is much more complicated, but it can be carried out in a similar way, and it leads to information of the same type, but in more detail. The electronic bands of homopolar molecules often exhibit vibrational and rotational fine-structure when the corresponding infra-red spectra themselves do not appear, and in these cases they are of particular value.

Typical results of the analysis of molecular spectra, which are described below, will illustrate its value in stereochemistry, but two other results of a different and more general character may be mentioned The fine-structure in molecular spectra is produced by the existence of molecules with small differences in energy. It will therefore also indicate the existence of molecules with slightly different moments of inertia. In this way, the presence of two isotopes doubles all the lines, and the intensity of each component of such a doublet is a measure of the concentration of the corresponding isotope. The displacements are too small to be observed in the pure rotational spectrum, but are evident in the vibration-rotation and electronic spectra, *e.g* the spectrum of HCl contains doublets due to the two isotopes of chlorine, whilst two new isotopes of oxygen, one of carbon, and one of nitrogen have been identified by this method. The other discovery of this type was that of the existence of two forms, known

as ortho- and para-, of the hydrogen molecule, whose energies are slightly different from one another.

For a more detailed account of the theory of molecular spectra and their applications, reference should be made to one or other of the works mentioned in the Bibliography (page 39), but a summary for one or two simple molecules will now be given. The CO_2 molecule must be of the form of a straight line, and the separation C to O is almost the same as in CO, whilst the character of the C to O bond appears to remain the same in both compounds. SO_2, on the other hand, is a " bent " molecule, with an angle between the two S to O bonds of approximately 120°. This agrees with evidence obtained from other sources, *e.g.* the dipole moment. H_2O is also " bent," with an angle *about* 105°, although this value is not yet definitely fixed. NH_3 has the form of a flat pyramid, and CH_4 that of a tetrahedron, the dimensions being known in each case. The spectrum of *acetylene*, C_2H_2, is exactly that which would be expected from a diatomic molecule, and the analysis fully agrees with a linear formula $H-C\equiv C-H$. The data for *ethylene*, C_2H_4, are not so complete, but suggest that all six atoms lie in the same plane, as would be expected on chemical grounds.

By collecting data of this kind, it has become possible to attribute structures to, and to determine the strengths of the bonds in, a large number of simple compounds. One of the interesting results has been to show that the nuclear separation of the atoms forming a radical remains the same when the radical is built into a molecule, so that " radicals are bricks which retain their dimensions in their stable compounds " (Mecke).

Continuous and Predissociation Spectra.

It was mentioned in Chapter I that the electronic absorption bands of some molecules become " continuous " as shorter wave-lengths are approached. Very careful examination has failed to reveal any sign of structure in these continuous regions. An explanation of this is that as the vibrational quantum number increases, the energy of vibration becomes so large as to break down the chemical bonds between the atoms. If a quantum of energy larger than a certain limiting quantity is absorbed, therefore, the molecule will break up, when the ordinary quantum theory of the absorption ceases to apply. If this is so, the limiting energy for the fine-structure spectrum should be the same as the heat of dissociation of the molecule. It must be remembered that the molecular fragments produced may not at first be in their lowest energy states—they may be " excited "—and allowance must be made for this excess of energy. When

this is done, however, in many cases very good agreement is obtained. Thus, the limiting wave-length, or " convergence point," in the spectrum of iodine, 4995 A, corresponds to an energy of 56,950 calories per gm. mol. From spectroscopic evidence, it can be deduced that the energy required to excite an iodine atom is 21,650 cals. per gm. atom. If the iodine molecule is dissociated into one normal and one excited atom, the heat of dissociation should be $56,950 - 21,650 = 35,300$ cals. per gm. mol. The heat of dissociation obtained thermochemically is 35,100 calories, so that the explanation given above appears to be valid ; and it is also confirmed from other spectroscopic evidence [1]

A related phenomenon was observed by Henri in 1924. In the electronic spectrum of sulphur vapour, rotational fine-structure does not appear in all the vibrational bands, but at wave-lengths below a certain limit, these bands appear to be diffuse. A similar effect has since been identified in many other spectra, and the systems of diffuse bands are known as " predissociation spectra." They are sometimes followed by continuous absorption, and sometimes by further fine-structure bands. The accepted explanation is that when the molecule absorbs more energy than is required for dissociation, this energy may be redistributed, causing excitation as well as dissociation. If the act of dissociation takes place in less time than is required for the molecule to complete a rotation, the rotational energy ceases to be quantised, and the corresponding fine-structure disappears. The frequency at which the predissociation spectrum begins clearly sets an upper limit to the heat of dissociation of the molecule.

The Raman Effect.

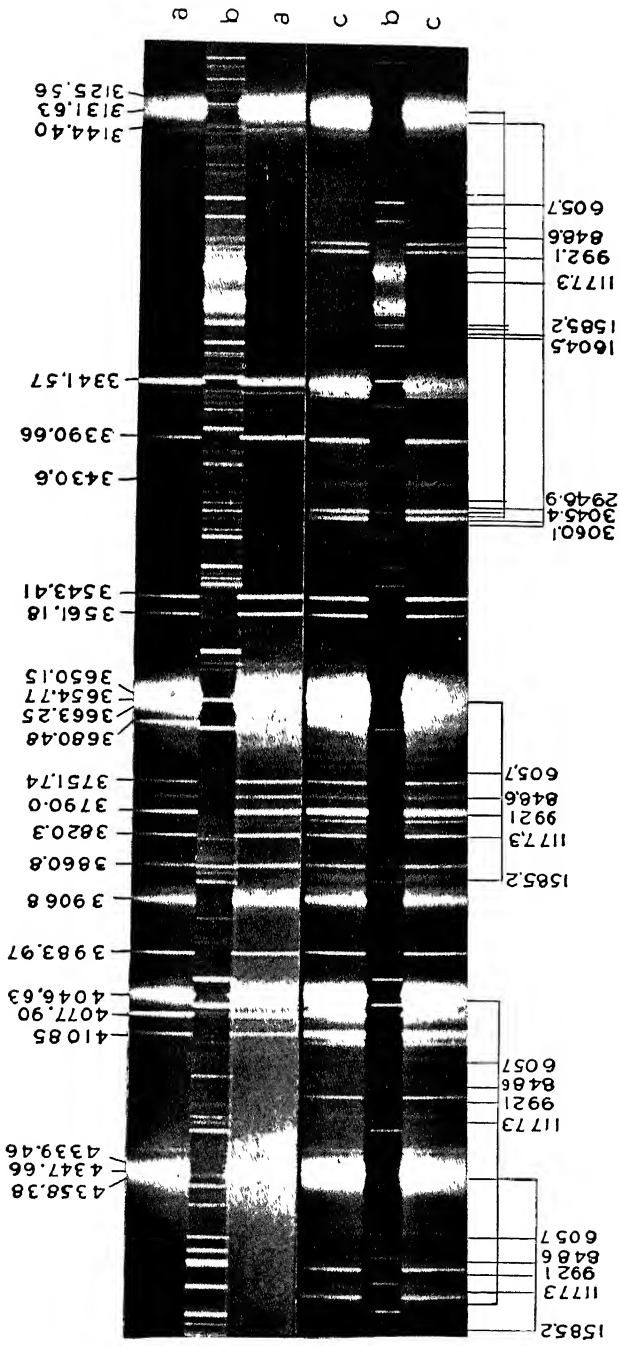
An effect which is closely related to molecular spectra was discovered by Sir C. V. Raman in 1928, using " a quartz spectrograph of the smallest size made by the firm of Hilger." [2] He observed that when a liquid is irradiated with monochromatic light, it scatters not only light of this same frequency, but also other frequencies slightly displaced from it. This change of frequency, to which the name " Raman Effect " has been

[1] The wave-length of the convergence point is converted into heat of dissociation as follows —The quantum Q required to dissociate a single molecule is related to this wave-length by the equation $Q = h\nu = hc/\lambda$ If the number of molecules in a gm. mol. (Avogadro's Number) = N, then the energy of dissociation of a gm mol = NQ, and this divided by the mechanical equivalent of heat, J, gives the heat of dissociation $= NQ/J = Nhc/J\lambda$ Putting in the usual values for the various constants, $N = 6\,062 \times 10^{23}$, $h = 6{\cdot}547 \times 10^{-27}$ ergs secs , $J = 4\,185 \times 10^{7}$ ergs per cal, the heat of dissociation $= 2\,845/\lambda$ (or $2{\cdot}845 \times 10^{8}/\lambda$ in Angstroms).

[2] Raman, Nobel Lecture on " The Molecular Scattering of Light "

PLATE VI

To face page 36



Spectrum of mercury light scattered by benzene (C_6H_6) made with a quartz prism spectrograph

a = spectrum of incident Hg light, b = Cu arc comparison spectrum, c = spectrum of scattered Hg light. Wave-lengths of Hg lines above, shifts of Raman lines below in wave-numbers.

FIG. 14.

PLATE VII

To face page 37

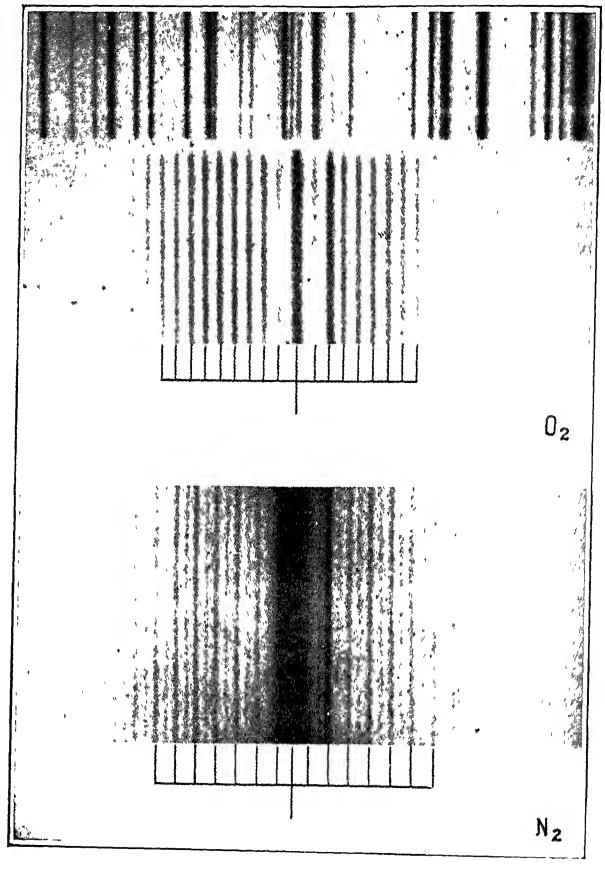


FIG. 15

given, has been found to be a very general phenomenon, and it has been measured for a large number of substances in all three states of aggregation. The wave-length of the scattered light is generally more than that of the incident radiation (there are exceptions) but the characteristic feature of the phenomenon is that the *difference in frequencies* of the incident and scattered light is generally equal to the frequency of an inter-atomic vibration as determined from the rotation-vibration spectrum of the compound. The change of energy is thus that which is required to raise the molecule from one vibrational state to another, and the lowest "Raman frequency"[1] observed would be expected to be that of the "fundamental" vibration (see p. 33). This is in fact found to be true in many cases, whilst for some substances, Raman lines have been observed which correspond to rotation frequencies, and even to electronic frequencies. An interesting feature is, however, that Raman spectra are observed with homopolar molecules, which do not exhibit the ordinary vibration spectra, so that the fundamental vibration frequency can then be found independently in this way. It has been established that definite linkages in organic compounds, *e.g.* C – H, C – C, C = C, C = O, etc., give rise to characteristic Raman frequencies in all their compounds, so that the Raman effect is also available for use in problems concerning molecular structure.

Fig. 14 represents the Raman effect in benzene excited by the mercury spectrum and recorded on an E 2 Quartz Spectrograph. On it are marked the Raman lines as excited by various lines in the spectrum of the irradiating light. It is taken from the work of Langer and Meggers[2] who state that good exposures could be obtained in several minutes, while those used for measurements were from fifteen minutes to two hours in order to observe the weaker lines. Fig. 15 shows the rotation Raman spectrum of oxygen and nitrogen under pressure investigated by Rosetti.[3] Exposures of 24 to 100 hours were given with an E 1 Spectrograph, using the λ2536 line for which the dispersion of the instrument is 3 A/mm.

The Absorption Spectra of Organic Compounds at Very Low Temperatures.

It was pointed out in Chapter I that the absorption bands exhibited by a liquid or a solution are continuous on account of the "blurring-out,"

[1] By "Raman frequency" is meant the frequency which absorbs that energy which is not scattered. If ν_i = incident frequency and ν_s the scattered frequency (Raman line), the Raman frequency ν is defined by $h\nu = h(\nu_i - \nu_s)$.

[2] *Bureau of Standards Journal of Research*, **4**, p. 711 (1930)

[3] *Zeits. f. Physik*, Vol 61, p. 598 (1930)

as a result of intermolecular disturbances, of the fine structure, which is revealed in the same bands when the spectrum of the vapour is examined. At very low temperatures, such as those of liquid air or of liquid hydrogen, however, not only is the thermal agitation of the molecules much reduced, but the modes of vibration and rotation of the individual molecules are simplified, and it has been found that the broad bands of the liquid then begin to break up into finer bands resembling those of the vapour.

An interesting example is the case of benzene, examined by Kronenberger.[1] The six bands forming the characteristic ultra-violet absorption spectrum of liquid benzene (Fig. 25) are well known to correspond to *groups* of fine-structure bands in the spectrum of the vapour. At the temperature of liquid hydrogen, the absorption bands of the benzene crystals also break up into groups of bands which exhibit considerable regularities in separation and intensity, and which can be analysed in the same way as the fine structure bands of the vapour. The spectra of benzene derivatives were also shown to be affected in a similar way.

Conant and Crawford[2] have examined the absorption spectra of solutions of porphyrins and other coloured compounds at liquid air temperatures, and find that the widest porphyrin bands then break up into a system of narrow bands, while Hausser[3] has recently investigated the low temperature spectra of a series of compounds containing multiply-conjugated ethylenic bonds, and finds that they exhibit very important structural features.

The method has also been applied to substances of biological interest, and Fig. 5 shows the effect of cooling to liquid air temperature on the absorption bands of carotene. By this method Bowden[4] was able to show that irradiated carotene is not identical with vitamin A as the absorptions of the two substances at ordinary temperatures had seemed to suggest.

Investigations of this type at low temperatures are becoming more and more numerous, and the field of research thus opened up appears likely to produce results of very great value.

[1] *Zeits. f. Physik*, Vol. 40, p. 75 (1926); Vol 63, p 494 (1930).

[2] *Proc. Nat Acad. Sci.*, Vol. 16, p. 552 (1930).

[3] *Zeit. f techn. Physik*, Vol. 15, p. 10 (1934)

[4] *Nature*, Vol. 131, p. 582 (1933); *Proc. Roy. Soc.*, in publication.

BIBLIOGRAPHY

FOR more detailed accounts of the subjects which have been discussed in Chapters I-III, in addition to the references given in the text, the following books may be consulted. They also contain bibliographies of the original papers on these and related subjects.

Absorption Spectra of Liquids.

V. Henri, *Etudes de Photochimie.* (Gauthier-Villars.)

The Origin of Spectra.

R. C. Johnson, *Spectra.* (Methuen.)

Jevons, *Report on Band Spectra.*

The Infra-Red.

Rawlins and Taylor, *Infra-Red Analysis of Molecular Structure,* (Cambridge University Press.)

Molecular Spectra and their Applications. The Raman Effect.

Mecke, *Bandenspektren und ihre Bedeutung für die Chemie.* (Borntraeger.)

P. Debye, *The Structure of Molecules.* (Blackie.)

K. Freudenberg, *Stereochemie.* (Deuticke.)

Kohlrausch, *Der Smekal-Raman Effekt.* (Springer.)

Chemical Aspects.

S. Glasstone, *Recent Advances in Physical Chemistry.* (Churchill.)

Applications of Absorption Spectra.

Recent Applications of Absorption Spectrophotometry (Adam Hilger, Ltd.).

Photochemistry.

Griffith and McKeown, *Photo-Processes in Gaseous and Liquid Systems.* (Longmans, 1929.)

Bonhoeffer and Harteck, *Grundlagen der Photochimie.* (Steinkopff, 1933.)

In the International Critical Tables (published by the National Research Council of the U.S.A. in 1929), a very comprehensive bibliography of absorption spectra of organic and inorganic solids, liquids, and gases up to that date is given in Volume V, pp. 326-359. Absorption curves for a number of the more common substances including dyes and some alkaloids are reproduced on pp. 361-379. In Volume VII, p. 173, a bibliography of dye-stuff absorptions is given. Data for the transparency of standard light filters appear in Volume V, p. 271, and for the absorption by glasses in Volume II, p. 106.

APPENDIX TO PART I

THE ALGEBRAICAL EXPRESSION OF BEER'S LAW, AND ITS USE IN THE ANALYSIS OF ABSORBING SOLUTIONS

IT is the purpose of this Section to show how Beer's Law is employed in the analysis of solutions of two substances whose solutions have measurable absorption curves, provided that the absorption curves of the substances dealt with in any one problem are different from each other and of course provided that Beer's Law holds.

Beer's Law as was explained on page 17 may be stated: "The value of the extinction coefficient is proportional to the concentration of the absorbing material." Beer's Law as originally stated had no reference to mixtures, but it seems customary to apply the phrase and the notion to mixtures. It has long been known how to obtain by the application of Beer's Law, if one has a mixture of two absorbing substances whose absorptions are known for two wave-lengths, the proportions in which the two substances are present in the mixture. What follows is a very simple corollary of a procedure described by Vierordt [1]

The following nomenclature will be used:—

Let a_0, a_1; β_0, β_1; ... etc., be the extinction coefficients of mixtures α, β... for wave-lengths λ_0, λ_1 ... .

Let a_0, a_1 ... , b_0, b_1 ... , c_0, c_1 ... etc. be the extinction coefficients of the component solutions A, B ... for wave-lengths λ_0, λ_1 ... .

Let m_α, m_β, ... ; n_α, n_β, ... , p_α, p_β, ... ; q_α, q_β ... ; be the concentrations of solutions a, b, c, d ... in mixtures α, β, ... in terms of that of the standard solutions.

Two cases will be considered.

CASE I.—*A mixture of two solutions* A *and* B *whose absorption curves are known. It is required to find the concentration of each solution in the mixture.*

[1] See K Vierordt, *Die Anwendung des Spectralapparates zur Photometrie der Absorptionsspectren und zur quantitativen chemischen Analyse*—Tubingen, 1873, on page 51 Vierordt describes how he checked the method on a mixture of potassium permanganate and potassium dichromate

See also K Vierordt, *Die quantitative Spektralanalyse in ihrer Anwendung auf Physiologie, Physik, Chemie, und Technologie* -Tubingen, 1876; an account of work on absorption spectrophotometry.

One mixture only is required, whose spectrophotometric curve is found by measurement.

By Beer's Law :

$$\alpha_0 = m_\alpha a_0 + n_\alpha b_0$$
$$\alpha_1 = m_\alpha a_1 + n_\alpha b_1$$

from which $m_\alpha = (\alpha_0 b_1 - \alpha_1 b_0)/(a_0 b_1 - a_1 b_0)$

and $n_\alpha = (\alpha_0 a_1 - \alpha_1 a_0)/(b_0 a_1 - b_1 a_0)$.

It will be observed that to find m_α and n_α we only need to know the extinction coefficients of solutions A and B and of the mixture for two wave-lengths. The wave-lengths need to be selected with consideration in each specific problem, in order that high accuracy may be attained in the determination of m_α and n_α.

CASE II.—*Mixture of two substances* A *and* B, *the absorption curve of only one of these being known.*

If one makes observations on a number of such mixtures (α, β, γ etc.) in which the concentrations of the substances A and B are different, then one is enabled to determine the ratio of the concentrations of the unknown substance B [1] in any two of these mixtures.

As in Case I, we can write down two equations for a mixture :—

$$\alpha_0 = m_\alpha a_0 + n_\alpha b_0$$
$$\alpha_1 = m_\alpha a_1 + n_\alpha b_1$$

in which now m_α, n_α, b_0, b_1, are unknown, a_0, a_1, being known. Similarly we have two equations for another mixture .—

$$\beta_0 = m_\beta a_0 + n_\beta b_0$$
$$\beta_1 = m_\beta a_1 + n_\beta b_1$$

In these four equations, there are six unknowns :—

$$m_\alpha,\ n_\alpha,\ m_\beta,\ n_\beta,\ b_0,\ b_1$$

so that a complete solution is impossible.

Nevertheless, the form of the equations enables one to obtain one piece of useful information, namely the ratio

$$n_\alpha/n_\beta = \{\alpha_0 - (a_0/a_1)\,\alpha_1\}/\{\beta_0 - (a_0/a_1)\,\beta_1\}.$$

As an example of a problem in which this would be of use, we may mention ethocaine. This substance is hydrolysed slowly in aqueous solution. A direct application of the above would therefore allow one to investigate the progress of decomposition.

As a concrete case, we may consider the curves of Fig. 16. Curves 1, 2 represent the extinction coefficients of two mixtures, which consist of different proportions of benzoic acid and another substance, unknown.

[1] B may itself be a mixture, provided the ingredients be in constant proportion.

Curve 3 represents the extinction coefficients of a solution containing benzoic acid only.

Take $\lambda_0 = 2500$, $\lambda_1 = 2800$.

Then we can obtain from the curves :—

$$\alpha_0 = 0{\cdot}525 \qquad \alpha_1 = 1{\cdot}14$$
$$\beta_0 = 0{\cdot}395 \qquad \beta_1 = 1{\cdot}62$$
$$a_0 = 0{\cdot}565 \qquad a_1 = 0{\cdot}59$$

Substituting in the expression obtained :—

$$n_\alpha/n_\beta = \{0{\cdot}525 - (0{\cdot}565/0{\cdot}59)\,1{\cdot}14\}/\{0{\cdot}395 - (0{\cdot}565/0{\cdot}59)\,1{\cdot}62\}$$
$$= (0{\cdot}525 - 1{\cdot}09)/(0{\cdot}395 - 1{\cdot}55)$$
$$= 0{\cdot}565/1{\cdot}155 = 0{\cdot}489.$$

That is, the concentrations of the unknown substance in the two mixtures are in the ratio 0·49 : 1.

Beer's law, as applied to mixtures, also has applications in biological processes, as will now be described by a few illustrative examples.

A Mixture of Two Biological Substances when the Absorption Curve of each is Known.

This problem may present itself in different ways as will be clear from what is said above. If the selective absorptions of two substances occurring as a mixture do not overlap, their estimation is simple. It is when there is overlapping of the absorption bands that difficulty is met with. One substance may be present in such excess that the absorption of the other is obscured. An example of this may be found in blood plasma, where the selective absorption of the proteins entirely masks that of the uric acid.

By filtering the plasma through a semipermeable membrane, the proteins can be separated from filtrate containing the uric acid. Several methods of ultrafiltration through semipermeable membranes are available. The ultrafilter devised by Campbell Smith [1] was specially designed for the purpose of rapidly filtering undiluted blood plasma.

The uric acid may then be determined in the filtrate and the protein computed by difference from the absorption of the whole plasma.

Protein precipitants are inadvisable as they all show marked absorption in the region of the spectrum under examination.

Holiday [2] has shown that an ultrafiltrate of whole blood shows, besides a band due to uric acid, a second band at 2600 A which has not been identified but is probably due to adenine nucleotide and thioneine.

Calculations of ratios of two biological substances in a mixture from the composite absorption curves are unreliable except under very restricted conditions. If it is known that the solution is a simple mixture of two

[1] *Journ. Phys*, Vol. 65. *Proc. Phys. Soc.* (1928).

[2] *Biochem. Journ.* XXIV, 3, 619, (1930).

substances without any other absorbing impurity, such calculation is legitimate. But as this hardly ever occurs in biological fluids it should not be attempted except for very rough calculation. If the maximum absorption of the two occurs at the same wave length and we have some other criterion by which to measure total weight of absorbing substance, a simple calculation will reveal the proportion present of the two substances. For example, Campbell Smith [1] showed that, for the same nitrogen content, albumen gave only half the absorption of pseudo-globulin although the maximum absorption of the two occurred at the same wave-lengths. Therefore if the nitrogen content of a certain mixture of albumin and globulin be known and also the extinction coefficient, the proportion of the two present can be calculated. Smith gave the following formula:

$$\text{Albumin/Globulin} \quad (E_h \quad 1.2x) \; (0.8x \quad E_h),$$

where E_h = extinction coefficient of the mixture at the maximum of the absorption curve, x = total concentration of protein per cent.

This calculation can be applied to serum proteins as the absorption of the constituents is almost negligible, usually, however, a correction is made for the uric acid and pigment present. This might well prove of value as a rapid method for following the change in Albumin Globulin ratio both in pathological conditions and in experimental immunization.

Except for a case of this kind, calculation from composite curves should be avoided; the aim should rather be to separate the various absorbing constituents of a mixture and to estimate them separately. This at present is a problem of considerable difficulty but one which should not prove insuperable.

[1] *Proc. Roy. Soc.*, B, Vol. 104 (1929).

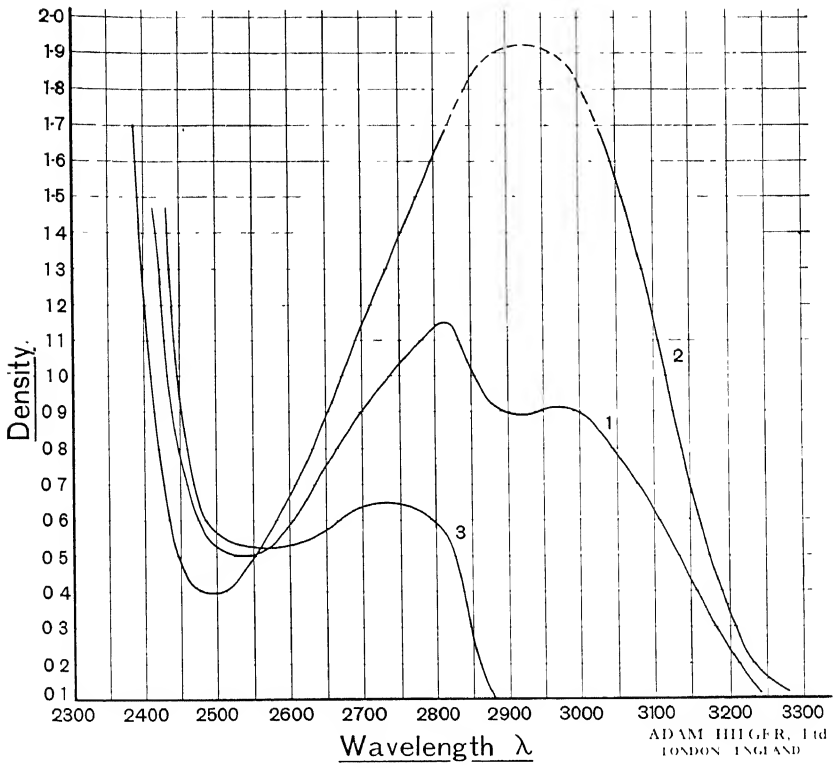

2·0
1·9
1·8
1·7
1·6
1·5
1·4
1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
Density.
2300
2400
2500
2600
2700
2800
2900
3000
3100
3200
3300
Wavelength λ
1
2
3
ADAM HILGER, Ltd
LONDON ENGLAND

FIG. 16.

PLATE IX

To face page 45

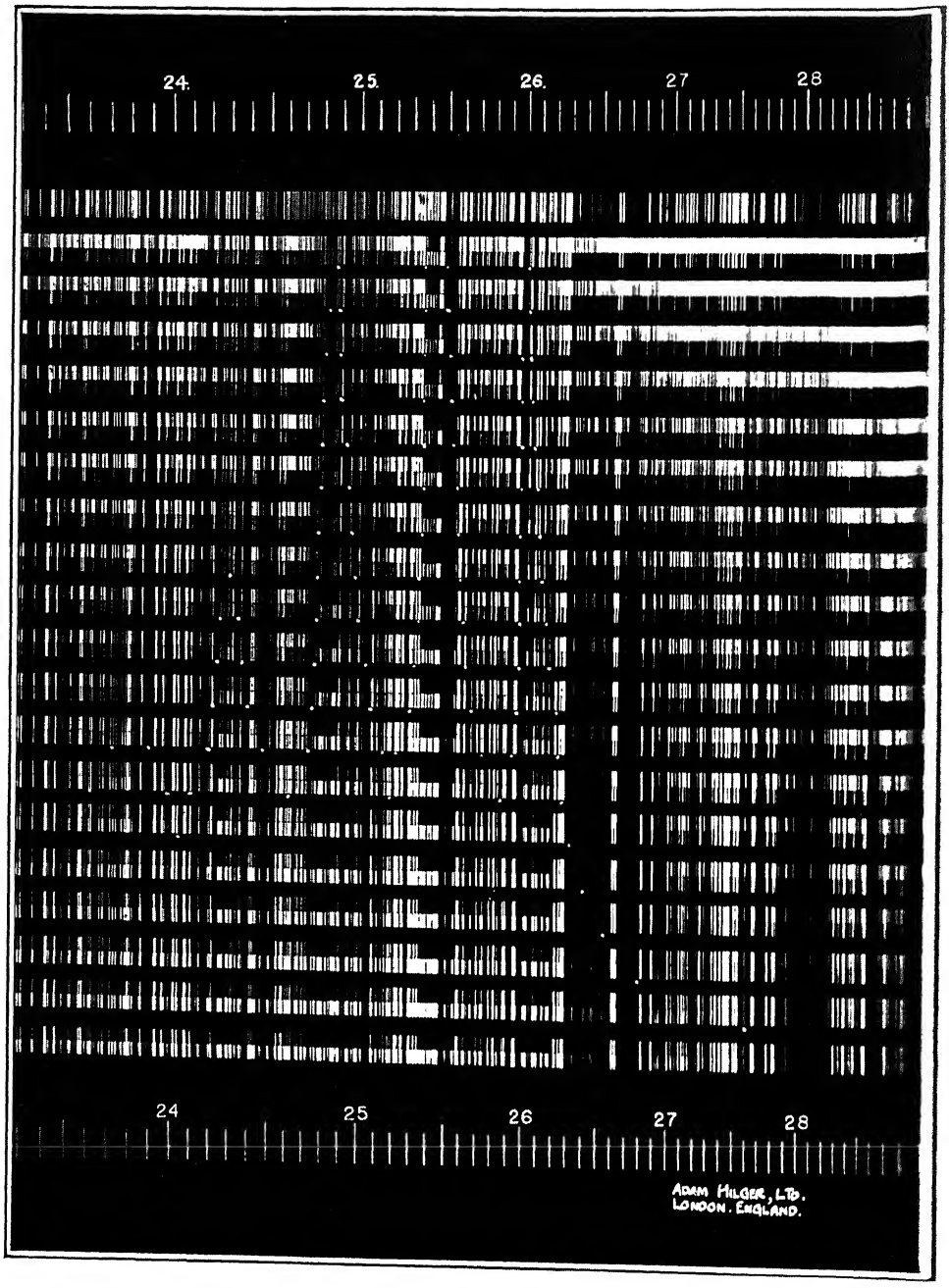


FIG 17

Absorption Photograph of Benzene (in hexane) taken with Spekker Ultra-Violet Photometer (H 237) and Medium all-metal quartz spectrograph (E 316) Length of tube 2 0 cms Minimum exposure 5 secs Enlargement × 2 of a portion of the photograph

PART II—THE TECHNIQUE OF ABSORPTION SPECTROSCOPY

CHAPTER IV[1]

THE CONDITIONS FOR SECURING ACCURACY IN ABSORPTION SPECTROPHOTOMETRY[2]

SPECTROPHOTOMETRIC measurements can be classified according to the nature of the problem to which they are to be applied. The applications of absorption spectroscopy described in the preceding chapter call for the measurement of:

(1) the absorption spectra of liquids over a wide range of wave-lengths;

(2) absolute extinction coefficients for a few, or over a narrow range of, wave-lengths to the highest obtainable accuracy;

(3) small changes in absorption;

(4) the wave-lengths or frequencies and intensities of the fine-structure in the absorption bands of a gas or vapour

To these we may add:

(5) the intensity of transmitted radiations.

The experimental problems involved in each case are different. Thus, in (1) the spectrum may consist of wide diffuse bands and the greatest accuracy in the measurement of wave-lengths and intensity may not be required. The speed with which records may be made over a wide range of spectrum and of extinction coefficients will probably then be the most important consideration. In (2), particularly if the results are to be applied to calculations, both extinction coefficient and wave-length must be measured precisely. For the detection of small changes in absorption

[1] *The greater part of this chapter is taken, with the permission of the Physical Society, from a paper by Twyman and Lothian,* Proc. Phys. Soc., *Vol* 45, 5, *No* 250, *p.* 643, 1933

Those to whom the subject of spectrophotometry is new, will find it well to read Chapters VI to IX, before this one.

[2] Some of the conclusions in this chapter were independently arrived at by von Halban and Eisenbrand, *Proc. Roy Soc* A, **116,** p 153 (1927)

(3), absolute values are not so important as sensitiveness combined with reproducibility of readings. In (4), highly accurate knowledge of the extinction coefficients of fine structure lines (in the present state of knowledge) is of secondary importance compared with the necessity for high resolving power and means of accurately measuring wave-lengths. Work coming in category (5) frequently (as in colorimetric work) requires no great dispersion. The present chapter discusses the accuracy to be desired in cases of these types; the instruments and arrangements appropriate for each purpose are described in subsequent chapters.

§ 1. THE PURPOSES OF SPECTROPHOTOMETRY

If we consider the applications mentioned above we find that they fall into two main categories: those in which we seek information as to the nature or state of the absorbing medium, and those in which our interest is directed towards the effect produced by a medium on the radiation transmitted or reflected by it. Thus we may measure the absorption in order to identify and estimate the absorbing substances, to decide some question of molecular constitution, or to ascertain what is taking place inside the atoms or molecules; and in all these cases it is the proportion of radiation which is *absorbed* which we desire to know accurately. On the other hand, we may use analogous methods to test media such as coloured filters or fabrics, or the glasses of which spectacles are to be made, in order to ascertain their effect on the transmitted or reflected radiation; and here we wish to know accurately what proportions of the radiations of various wave-lengths are *transmitted*.

In the first of these classes the function of absorption in terms of which it is appropriate to express our results is the Roscoe-Bunsen extinction coefficient K, which for any substance and wave-length is, according to Beer's law, proportional to the concentration of the absorbing material. Thus the proportional accuracy with which a substance can be characterized by its absorption follows that with which K can be determined, and is represented by $\Delta K/K$ where ΔK is the smallest measurable increment of K

A radiation, on the other hand, is best characterized by its intensity, and the proportional error of its measurement is represented by $\Delta I/I$, where ΔI is the smallest measureable increment of I, the intensity of the incident radiation.

These two classes of measurement require differing modes of attack if the highest accuracy is to be attained. The first is considered on pp 47 to 62, the second on pp. 62 to 65.

§ 2. THE CHARACTERIZATION OF A SUBSTANCE BY ITS ABSORPTION

THE DISPERSION SYSTEM, LIGHT SOURCE AND CELLS

The Dispersion System.

The points of an absorption curve where great accuracy is required will often be on the slope of the curve, so that if the measurements made on different occasions are to correspond we must ensure that the wave-lengths used are always the same, within the limit of the following condition : *The error of density due to error in wave-length setting should be less than the error in the photometric measurement of the density.* Thus, in making use of a continuous spectrum, an instrument of sufficiently high dispersion is needed ; and the steeper the curve at the point under consideration, the greater the dispersion desirable.

To take an illustration : in the use of a visual spectrophotometer for determining small percentages of carboxyhaemoglobin in blood, useful accuracy can only be attained by making measurements near the two wave-lengths 5600 and 5770 A. At the latter wave-length the spectrophotometric curve of carboxyhaemoglobin is so steep that very narrow slits must be used in order to get a spectrophotometric measurement which has useful meaning.

In consequence of the low intensity of the light transmitted at large densities, it may sometimes be desirable to increase the width of the collimator slit. Where the source has a line spectrum this slit can be opened considerably without sacrifice of purity of spectrum, for one can then use the same lines on each occasion so that the above condition as to wave-length setting is automatically fulfilled. With a continuous-spectrum source, however, one must effect a compromise between the best light-intensity and the desired purity of spectrum.

In studying fine-structure the dispersion is of pre-eminent importance, and the nature of the spectra precludes the use of photo-electric methods of measuring the absorption. With photographic methods the largest prism instruments ordinarily available may still provide too small a resolution for the densities of individual fine-structure lines to be measured accurately. At the present time interest in this field is centred chiefly on the wave-lengths of these lines, and one is satisfied if these are clearly resolved. To do this for fine-structure in the visible and ultra-violet the quartz spectrographs of focal length 170 cms. (E 1, E 384) suffice, supplemented where necessary by the addition of the glass train for the visible spectrum (E. 52).

The absorption of the fine-structure lines can be deduced by measure-

ments of a microphotometric trace of the photograph, although the ordinates of such a trace are very complex functions of the extinction coefficient, involving the characteristics of the light source, of the photographic plate[1] and of the spectrograph slit.

Microphotometric measurement of wave-length. If in any spectrum it is necessary to deduce a wave-length from the microphotometer trace, it is usual to do it by interpolation between two known lines which are superposed on the continuous spectrum. Mercury lines have generally been used for this. The method presupposes a knowledge of the dispersion on the original plate, and an interpolation formula has to be used. The wave-lengths of fine-structure lines can generally be measured directly from the spectrogram, however, and then a comparison iron arc is used.

It is possible that workers in this field will not remain satisfied with a dispersion which just suffices to resolve the bands, but will seek such a resolving power as will permit point-to-point spectrophotometric measurements to be made—laborious though such a procedure would be. Large concave gratings have many disadvantages for absorption spectrophotometry, and the instrument maker will do well to explore the possibilities of making large liquid prisms. To realise such possibilities to the full, the temperature of such prisms will have to be thermostatically controlled.

For fine-structure in the infra red the Muller-Hilger Double monochromator with quartz or rock-salt prisms and lenses, and Hilger or Müller thermopile is recommended, or where very large dispersion is needed the prism grating instrument D 79 is suitable.

Range of Spectrum studied.

By the "infra-red" is meant those wave-lengths greater than about 1μ; by "visible" those from 8,000 A (1μ) to, say, 3900 A, while the "ultra-violet" is usually taken as extending from about 3900 A to the very sudden air absorption at 1850 A. Shorter wave-lengths than 1850 A are sometimes referred to as being in the Schumann (from 1850 to 1250 A) or Lyman (from 1250 to 600) regions. Fluorite and grating spectrographs are available for these regions, the latter penetrating even into the X-ray field (as far as 10 A), from which point X-ray Crystal Spectrographs are used.

[1] The importance of variation of intensity among the fine structure lines has already been realised, and interpretations are being sought and found in some cases. The intensity is, of course, a measure of the probability of the transition concerned and the wave-mechanics, as it develops, will certainly work out along lines of probability: *i.e.* of intensity. The measurement of intensities may thus become vital in a very short time. The way in which intensities of emission lines (and hence absorption lines) are deduced from microphotometric traces, is dealt with by Ornstein, Moll and Burger, *Objektive Spektralphotometrie* (F. Vieweg, 1932).

Although most of the remarks in this chapter are general in character, there is throughout the tacit assumption that we are dealing with spectrophotometry in the range from 1360 to 160,000 A.

The range of spectrum to which spectrophotometry has hitherto been applied other than sporadically, extends in the ultra-violet only to 1850 A. Not only quartz but air itself is opaque to rays of wave-lengths less than 1850 A, beyond which, therefore, vacuum instruments must be used. A vacuum spectrophotometer (H 345) has just become available giving accurate measurements of extinction coefficients down to 1360 A.[1] In the infra-red beyond 10μ [2] it becomes difficult to obtain a light source of adequate energy, while beyond 21μ (accessible with Sylvine prisms) none of the ordinary optical materials is suitable for prisms The region from (and including) the visible to 1850 A is accessible to quartz spectrographs and quartz spectrometers. Owing to the reduced dispersion of quartz in the visible region glass spectrographs and spectrometers are more suitable there.

Light Source.

In all methods of visual or photographic spectrophotometry in which comparison is made of the intensities of two closely adjacent spectra either viewed or taken simultaneously, it is essential that the relative intensities of such spectra should depend only on the absorption of the substance which is placed in one beam, and the deliberate variation of intensity which is imposed on the other beam.

An essential condition for accurate spectrophotometry of this type is that the rays which have passed through the middle of the slit (which corresponds with the point of the spectrum where comparisons of intensity are made) should pass through the spectrograph *without any vignetting*. That this condition is fulfilled can be tested by looking through the spectroscope or spectrograph (in the former case after removal of the eyepiece), using a wide slit.

In such types of instrument the light reaches the spectroscopic apparatus through two separate optical paths, so that if the adjustment of that part of the apparatus which is external to the spectrograph fails to be ideally perfect, the two beams of light may fail to be quite coincident in their passage through the spectroscopic apparatus—for example, in the Hilger Nutting Spectrophotometer (see page 99) an image of the light source is formed in the neighbourhood of the dispersing prism of the spectro-

[1] See *also* the Publisher's Catalogue E which includes prism, grating, vacuum and X-ray Spectrographs dealing with the spectrum from 13,000 A to 0·1 A.

[2] For infra-red Spectrometers see the Publisher's Catalogue D.

meter and in general there will be two such images not quite coincident.

If then the light source is out of centre, one of the images may be vignetted even if the other is not, or one may be vignetted to a greater extent than the other. In this way the relative intensities of the two beams may be altered and error in the measurement result.

It is thus necessary to have the light-source *small enough to be included within the aperture of the spectroscope or spectrograph.* But in order to obtain high accuracy large densities must be used, for these are needed for high accuracy in the characterization of substances by means of their absorption, and consequently as strong an intensity of illumination as possible must be obtained. Only by filling the aperture with light can the intrinsic brightness of the light-source be fully utilized. Thus the condition stated above in italics is antagonistic to the condition for high accuracy in the measurement of extinction coefficients.

Were it possible to obtain a light-source of great intrinsic brightness which at the same time is entirely uniform in radiation per element of its radiating area, the best course to adopt would be to produce an image which would entirely fill the aperture at all wave-lengths. Such a light-source, however, only exists in the form of an enclosed chamber within which the light-source is situated, and with such a means of illumination the intrinsic brightness is sometimes insufficient to give accuracy in those measurements of absorption with which we are now concerned. Thus the condition to be aimed at is that the trace of the light-source should pass through the spectroscope without being vignetted, but with no more margin than is needed to provide for any eccentricity that may be accidentally present.

Similar considerations are applicable in the methods of photographic spectrophotometry.

There are one or two miscellaneous points which should be referred to here. Whatever the form of spectrophotometer, measurements are made for radiations assumed to be monochromatic. Now such radiations may be impure in two ways, either by too wide a range of spectrum being included or owing to reflection and scattering the monochromatic radiation may be contaminated with stray light of other wave-lengths. Both these effects are liable to occur in using a monochromator in conjunction with a photoelectric photometer. Impurity of the second kind is in nearly all possible variations of the photoelectric method a factor which has a most important bearing on the accuracy obtainable. An example of the importance of this is given on page 158 of von Halban and Eisenbrand's paper (*loc. cit.*). To obviate this source of error it is therefore desirable to use a double instrument (*e.g.* D 107).

In every method it is, of course, necessary to have sufficient dispersion

(see above under " The Dispersing System "), but impurity of the second kind (scatter) is in the photographic method of very minor importance, since both the spectra are affected alike at the match point.

An essential difference between the photographic and photoelectric methods is that in all photographic methods the substance under measurement is placed in the undispersed beam, while in photoelectric methods it is usual to put it in the monochromatic beam. This is sometimes of importance with liquids which react quickly under irradiation, since in the former method the intensity of the light falling on the liquid is so very much greater. This objection to the photographic method is least important in the case of the Notched Echelon Cell (see page 75) as the complete exposure need only last a few seconds.

The accuracy with which Cells for Spectrophotometry should be made.

Visual and photographic measurements. The accuracy of the cells should be such that the departure from the nominal length of liquid should cause no measurable error. The least error in a density-measurement likely to be achieved in practice is probably $\pm$ 0·005 in the density reading. At a density of, say, 1·5, this is equal to 0·3 per cent. Thus the error in the cells should not be greater, for the best work than, say, one-half of this, *i.e.* 0·15%

Photoelectric measurements. As will be shown on p. 57, the best density to measure with a photoelectric null method is 0·4343. In measuring such a density a very much greater *sensitiveness* of measurement can be attained than with either visual or photographic methods. It may be doubted whether absolute measurements of density made by photoelectric null methods yet yield an accuracy in accordance with this degree of sensitiveness; but however this may be, in measuring a density of 0·4343, cells with an accuracy of $\pm$ 0·15% will not be responsible for an error greater than $\pm$ 0·00055 in the density-measurement. Not until an accuracy of density-measurement approaching this is desired and expected will any higher accuracy of cell become necessary.

The accuracy required in setting up the cell. Unless the cell is set every time perpendicular to the light beam, the length of light-path through the absorbing medium will vary. Suppose that the normal to the cell makes a small angle i with the incident light beam. If μ is the refractive index of the absorbing medium, it is easily seen that the fractional error δ in the path is given by

$$i = \mu\sqrt{(2\delta)}.$$

For instance, if $\mu = 1{\cdot}3$ and $\delta = 0{\cdot}0015$ then $i = 4°\ 6'$. Thus the cell should be set with its plane perpendicular to the light ray to within 4°.

With very little trouble, the cell can be set to 1°, so that the error due to this effect should be negligible although special attention to this point is required with short cells. With long cells (5 to 10 cms. for instance) another effect should be looked for—the beam would be displaced sideways, thus producing inequality of illumination in the two beams.

The length of cell should be chosen to obtain a density approximating to the optimum value for the method used, and different cells may for this reason sometimes be used with advantage for different portions of the same absorption band.

Effect of temperature. In some cases, absorption varies very considerably with temperature.[1] It is thus important in any particular case to ascertain by trial if such an effect is present, in which case steps should be taken to ensure that measurements are taken at a temperature sufficiently constant for any error introduced in this way to be negligible compared with other sources of error.

THE VARIOUS MEANS OF PHOTOMETRY

We must now consider broadly the means of photometry which are available, and what is the best way to employ each in order to determine extinction coefficients accurately. The means we shall consider are the following: (*a*) photographed pairs of spectra examined visually; (*b*) photographed pairs of spectra examined by a microphotometer; (*c*) visual photometry; (*d*) direct measurement by an objective photometer employing photoelectric, selenium or photronic cells, thermopiles or bolometers.

(*a*) *and* (*b*) *Photographic means of photometry.* In photographic methods of spectrophotometry, in which two juxtaposed photographs of spectra are compared visually, the differences in density of blackening which it is possible to detect are of the order of, say, 0·06.

It has long been known that for neighbouring areas of a photographic plate and for a constant time of exposure, the density of blackening of the plate is related to the log of the intensity, I, of the incident light in the way shown by the curves of Fig. 18.

The ratio of the increase of the density of blackening to the increase of the logarithm of light intensity (*i.e.* the slope of the curve) is greatest on the straight portion of the curve, where it is specially denoted by the symbol γ. Thus, in making photometric measurements, a plate and method of deve-

[1] Mukerji, Bhattacharji and Dahr (*J. Phys. Chem.* **32**, 1834 (1928); *J Phys. Chem.* **35**, 653 (1931)). In the former paper a case is recorded where a 30° rise of temperature caused the density to increase about two and a half times.

lopment (which latter is also very important) giving a large γ is desirable,[1] as at *a*, Fig. 18. On the straight portion of the curve the smallest detectable change in $\log_{10} I$ is obviously given by the smallest detectable difference in density of blackening divided by γ. If a plate having a value of γ equal to 6 be used, then it should be possible to detect a difference in the density of the absorbing substance equal to 0·06÷6, or 0·01, and this is confirmed by experiment.[2]

With a large γ the range of correct exposure (given by the straight portion *cd*, Fig. 18) is smaller, so that if a plate of large γ be used one must adjust the exposure of the part of the spectrum where a match is being

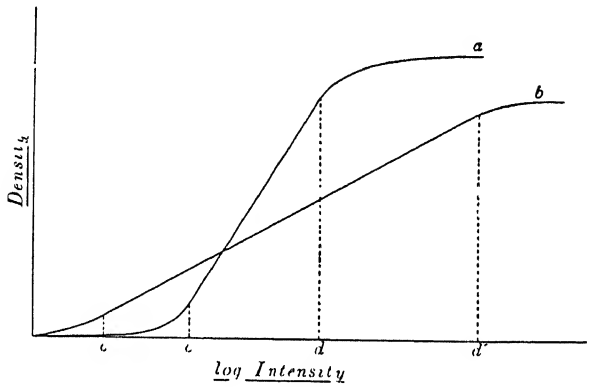


FIG. 18.

made in accordance with the spectral distribution of the light-source and the sensitivity of the emulsion to different wave-lengths, in such a way that one is working on the portion *cd* of the curve.

For preliminary surveys one will require a plate with a large latitude *c' d'*, Fig. 18; that is, a plate with small γ.[3] In this case, however, the measurement of densities will be much less accurate.

[1] The table in the Appendix shows that the most suitable Ilford plate would be the panchromatic half-tone The Eastman plates recommended are (*a*) Panchromatic Wratten M (III-B), or alternatively III-F (*b*) Where panchromatism is not desired, Wratten Metallographic or III-H. (*c*) For the ultra-violet region, III-F, III-H, or III-O, treated by the ultra-violet-sensitizing method. The most suitable developer for all the above plates is that listed by Eastman as D. 19 For brevity we shall subsequently refer to the combination of plate and development simply as " plate "

[2] Twyman and Lothian, *loc cit.*

[3] The most suitable Ilford plate here is the Hypersensitive Panchromatic. Eastman Kodak recommend (*a*) Panchromatic —I-F (*b*) Non-panchromatic—Eastman D.C. Ortho (I-H) or Wratten Hypersensitive (*c*) Ultra-violet—I-F.—U.V. Sensitive. A suitable developer in this case is D-76-C.

The contrast of a plate depends on the developer used, being at a maximum with alkaline quinol, and by increasing the concentration of both quinol and potassium hydroxide, the contrast may be still further increased. There is, however, a limit to the concentration of the developer, since the oxidation product of quinol—quinone—produces severe staining of the plate.

Considerable research has been carried out on the methods by which he development process may be made quite uniform over the whole area of the plate. In practice, it may be stated that no matter how complete the agitation may be, the edges of the plate will be developed more quickly than the centre. Over the major portion of the plate, however, the question scarcely arises when development is allowed to continue until γ_∞ is approximately reached (see later) with normal agitation by rocking the developing dish. Measurements near the edges of the plate are liable to considerable error.

The time of development also plays an important part in the production of contrast. With increase of time of development γ increases exponentially to a maximum value γ_∞. The minimum time of development required to produce an approximation to γ_∞ is different for each developer.

In the development process it is found that surrounding the dense portions there is a region where development is incomplete (the Eberhard effect) which is caused by the local production of a large quantity of bromide which acts as a restrainer. Care is necessary, therefore, to provide adequate agitation, but it is found that if development is allowed to proceed for a sufficient time to produce the greatest contrast (usually about 4-5 mins.) with ordinary agitation, the Eberhard effect is unnoticeable.

It should be noted that the contrast varies with wave-length, being at maximum in the visible, and decreasing with decreasing wave-length. The actual curve is to be found in G. R. Harrison's paper (*Journal of the Optical Society of America*, Oct. 1925).

A list of γ values for various plates and developers, as given by the makers, is given in Appendix I.

It has been held that by visual examination of such photographs as those in question, densities can be measured to within 0·02 to 0·04, *independently of the absolute value of density*.[1] As we have seen, this considerably underestimates the accuracy that can be attained, but in any case it remains true that in order that the percentage error in the measurment of the density should be small in photographic spectrophotometry, *the density itself should be large*. It has in the past been very usual to work

[1] See H. von Halban and J Eisenbrand, *Proc. Roy. Soc* A, 116, 154 (1927).

with densities not exceeding 1·5, but this seems frequently to have been due to the limitations of the instruments available rather than to any more fundamental reason.

As will be seen later the " Spekker " ultra-violet photometer enables high densities to be measured with a greater accuracy than the sector photometers.

In the use of large densities with a rotating-sector photometer the large number of long exposures required will cause a serious increase in the time required to obtain an absorption curve. In addition, the accuracy in making such sectors does not usually justify the measurement therewith of densities greater than 1·5. In using the notched echelon cell,[1] however, only one exposure is required to obtain a number of readings so that the measurement of relatively large densities does not take unduly long.

To take an example, if in the use of an echelon cell of greatest length 1 cm. the density of liquid measured is 0·3, giving extinction coefficients from 0·3 to 3 and requiring an exposure of about five seconds, we may get an error of from 7 per cent. in the measurement of the density, (corresponding to 0·02 error of density), and a like error in the values of the extinction coefficients. If, however, the density of liquid measured is 1·5, giving extinction coefficients from 1·5 to 15 and requiring an exposure of about 1¼ minutes, the error will not be more than 1½ per cent. Thus the echelon cell is at its best in conjunction with high extinction coefficients.

The employment of photoelectric instead of visual means of comparing the pairs of spectra does not alter the above conclusions, although it gives a considerably higher accuracy in determining the match points. A simple form of photoelectric microphotometer has been designed for this work (H 364).

Dobson [2] has used a method of photometry to which the same fundamental principles apply. In this a neutral-tint wedge is placed in front of the plate or the slit of the spectrograph, the gradient of the wedge being parallel to the length of the slit. A standard source is photographed on each plate as well as the spectra under investigation, and the points at which the density of blackening of the spectrum is equal to that of the standard are determined by means of a photometer. An objection to this method is that points to be compared are not closely adjacent on a plate, so that possible irregularities of development and plate are in this method a source of error.

[1] See p 75. For a fuller account of the cell, see Twyman, Spencer and Harvey, *Trans Opt Soc*, **33**, 37 (1931-32), and Twyman, *Proc. Phys. Soc.*, **45**, 1 (1933).

[2] Dobson, Griffiths and Harrison, *Photographic Photometry* (1926). It is assumed that the cell is made or measured with sufficient accuracy for the errors in thickness to be negligible. See p. 76.

(*c*) *Visual spectrophotometry.* The percentage error in a density-measurement made by a match with the eye is smaller the higher the density,[1] as in the photographic method, within the following limitations :

(1) The sensitivity of the eye to a given small percentage difference in brightness falls off at low intensities. This is specially noticeable at the violet end of the spectrum where the eye, the light-source, and the increased dispersion of the spectrometer act together in reducing brightness. This can be mitigated without unduly sacrificing the purity of the spectrum, by using for the violet a wider front slit than in other parts of the spectrum. Very great advantage is derived from placing an appropriate filter immediately in front of the slit, or outside the eyepiece in order to absorb the scattered light of wave-lengths which are transmitted freely by the substance under test.

(2) With some visual spectrophotometers it is found possible to obtain an accuracy of about 0·005 in density-measurements by taking a number of observations, this residual error being due to the limit of sensitivity of the eye. For densities of about 2·0 the scale usually becomes so cramped with forms employing polarising prisms that there is in addition an error in reading the scale of about the same magnitude (0·005). For this reason densities of between about 1·5 and 2·0 give the best accuracy.[2]

For the most accurate work a density-reading is accompanied by a reading of the zero for the particular wave-length in question.[3] The accuracy of density-measurement can then be increased by taking readings with the absorbing medium first in one beam and then in the other, the density being half the difference between the two readings. By reading on the opposite side of the zero instead of on the zero itself one obtains twice the accuracy with the same number of readings, *i.e.* with the same expenditure of time.

(*d*) *Objective means of photometry.* A photoelectric method[4] may also be used to measure the absorption of a medium. The sensitivity of such an arrangement is limited by the smallest change in light-intensity which can be measured. In a given arrangement suppose $\Delta\theta$ to be the smallest detectable change in the value of the deflection θ of the galvanometer or

[1] It easily follows from the Weber-Fechner law that the smallest detectable change of density (Δd) has a constant value independent of the absolute value of density.

[2] The scale of the spectrophotometer (H 181, see p 99) described by Dowell in *J. Sci. Instr*, 8, 382 (1931) and *J. Sci Instr*, **10,** 153 (1933), which follows a $\tan^4$ law, enables densities above 2 to be used with advantage. See p 99.

[3] An error in the zero becomes very serious at high densities

[4] In which it must be understood that the other objective methods mentioned are included.

other indicating instrument. Then ΔI, the smallest detectable change in light-intensity, is given by $\Delta I = k\,\Delta\theta$, k being a constant for the particular arrangement. Thus, in photoelectric measurements with a given apparatus ΔI has a constant value independent of the intensity I of the transmitted light, in contrast to the eye and photographic plate, for which, within limits, $\Delta I/I$ has a constant value.

The condition for greatest accuracy in absorption-measurements is that the percentage accuracy in the measurement of an extinction coefficient shall be a maximum. This implies a minimum value for $\Delta d/d$, where d and Δd are, respectively, density and the smallest detectable change in density.

If I_0 is the intensity of light incident on the absorbing medium and I the amount transmitted into the measuring apparatus, then the density is given by

$$d = \log_{10} I_0/I \qquad \ldots\ldots(1).$$

On differentiation this becomes

$$\Delta d = -\log_{10} e.\ \Delta I/I \qquad \ldots\ldots(2).$$

$$\therefore\ \Delta d/d = -\log_{10} e.\ \Delta I/Id.$$

$$= -\log_{10} e.\ \Delta I/I_0 d.10^{-d}. \qquad \ldots\ldots(3).$$

To find the optimum value of d we must find when expression (3) has a minimum value, *i.e.* when $d.10^{-d}$ from (3) has a maximum value.

Thus, differentiating and equating to zero we get $d = 1/\log_e 10 = 0{\cdot}4343$, so that the best density to use is 0·4343. We can see how critical this value is by examining the following values of the expression $d.10^{-d}$ from equation (3).

d.	1	0·8	0·43	0·2	0·1
$d.10^{-d}$	0·100	0·127	0·159	0·126	0·079

Thus we may use densities between 0·2 and 0·8 without any considerable loss of accuracy. The above remarks apply in general to photoelectric methods, whether null or deflection methods. But in any particular instrument other features of design might cause a departure from this condition.

There is no doubt that photoelectric methods give much greater sensitivity of discrimination in intensities than the eye. The balance of published evidence seems still on the whole to indicate that so far as absolute measurements are concerned, visual and photographic methods are more to be relied upon than photoelectric ones

There is no essential reason why this should be so,[1] though there are sources of error which are more likely to escape attention in the last named

[1] Indeed the present writer is of the contrary opinion. See p. 84

class. One of these is stray radiation. Stray radiation intense enough to be of importance in visual or photographic spectrophotometry can be seen, or photographed, as the case may be, when steps may be taken for its avoidance. When a photoelectric cell is the radiation detector, such discrimination is difficult.

Even if all causes of error be removed, however, one is not necessarily in a position to realise in absolute measurements an accuracy commensurable with the sensitiveness, for besides the electrical circuit, the photometric devices (wedges, sectors, etc.) may also impose limits on accuracy.

A Photoelectric Spectrophotometer (H 267) which attains a high measure of accuracy will be found described on page 81.

We are now in a position to summarise our conclusions as to suitable methods for various types of work and the accuracy which can be attained with them.

APPLICATION OF THE ABOVE PRINCIPLES, AND COMPARISON OF THE VARIOUS INSTRUMENTAL METHODS IN SPECIFIC EXAMPLES

The measurement of extinction coefficients in the ultra-violet. We shall assume throughout under this heading that we can increase the density of the substance under test to any degree that may be desirable, which can usually be done without difficulty by taking a more concentrated solution or a greater thickness of the absorbing substance.

(*a*) Photographed spectra examined visually. With a quartz spectrograph of the Hilger E 3 type and the usual sparking set,[1] photographic plate and development,[2] and tungsten steel electrodes (F. 406) with 4 mm spark gap, an exposure of 0·5 sec. suffices to give workable blackening of the photographic plate at wave-lengths from 4600 to 2100 A. The accuracy of matching of spectra thus produced has been found by us to correspond to a density-difference of 0·01 in the absorbing substance.[3] From this we find that for a measured density of 1, with exposure-time 5 sec., the accuracy $\Delta d/d$ is 0·01. For a density of 2 the figures are 50 sec. and 0·005 respectively.

(*b*) If the match points of a plate are determined by microphotometer —instead of by eye—the sensitivity is increased several times. At any point, the eye will in general perceive a match over a small range of wave-lengths. If the gradient of the absorption curve at the point is not too great there will be a number of lines in this range, and the microphoto-

[1] As described by Twyman and Hitchen, *Proc. R. S.*, A, **133**, 74 (1931), but without inductance in the spark circuit.

[2] Ilford panchromatic half-tone with developer No. 2 on the list on p. 114.

[3] With such photometric apparatus as that described on page 72.

meter will find a match on only one (say) of these. In this way it has been found possible to increase the accuracy of matching *thirty times*.[1] Where there are insufficient lines for the microphotometer to match on any one line, it may be possible by direct interpolation to determine a point of match between two adjacent lines.

(*c*) Direct measurement by objective photometer (with photocell), using the optimum density. It has been stated [2] that under favourable conditions the sensitivity may be 10^2 times that of the photographic method in the determination of an extinction coefficient. Using a plate of high γ for the photographic method would reduce this to from 30 to 50 times in favour of the photoelectric photometer, provided fairly wide slits can be used. The method is very much slower than the photographic, except in the special case where only a few readings at specified wave-lengths are needed. The absolute accuracy is limited as a rule by that of the sectors, wedges, etc., by means of which a match is made. A powerful monochromator is needed to secure purity combined with sufficient light. The method is, however, indispensable where very small changes in absorption are to be observed. Such work is of great importance in physical chemistry including as it does the detection of changes which a system may undergo under definite conditions, such as equilibrium displacements and the effect of various other factors which influence light absorption, and the investigation of the validity of Beer's law.

There is probably a greater variation of sensitivity between different types of photoelectric instruments than of photographic instruments, and the most important limiting factor in the former is the method of measuring currents. There is probably little to choose in this respect between electrometer methods and valve amplification. With the recently developed "electrometer valves" the valve methods can probably be raised to a greater degree of sensitivity than the electrometer methods, but up to the limits of their sensitivity electrometer methods are easier to use.

Measurements in the visible region. In the visible region visual, photographic and photoelectric methods are available, and the choice of a method depends on what accuracy is required, whether time is limited, and the nature of the absorption curve to be measured.

The visual method is the one most usually employed. With polarisation spectrophotometers it is possible to make accurate readings up to of densities nearly 2·0 with forms obeying a $\tan^2$ law, or somewhat higher with a $\tan^4$ law.

[1] The figures can be regarded as representing an increase of sensitivity but not necessarily of absolute accuracy, which latter will depend on the uniformity of cells, sector plates, etc

[2] H. von Halban and J. Eisenbrand, *Proc. Roy. Soc*, A, 116, 162 (1927)

The photographic method is often quicker, and the whole of the spectrum may be studied on one plate. Reference to p. 64 will show that with the recommended Ilford half-tone plate the accuracy of measurement with the Spekker photometer is about 0·01 in $\Delta d/d$.

As regards the time required: for measurements at a number of wave-lengths to obtain a complete curve, the photographic method is quicker than a photoelectric measurement when readings have to be obtained and plotted wave-length by wave-length. The photoelectric cell, however, lends itself to automatic recording of spectrophotometric curves. So far as we know this has only been performed in the visible region,[1] but there is no reason why it should be so limited. This is the most rapid method of spectrophotometry available, as about half a minute is sufficient to produce a complete curve in the visible region. The sensitivity is found to be limited by the finite thickness of the recording line, among other factors. It is a type of instrument which is extremely useful in industry for comparison, but presents great difficulties if absolute accuracy is sought.

With a visual instrument a number of readings must be taken at each wave-length in order to obtain a mean result of high accuracy. This makes the method more lengthy, but the accuracy is then greater than is obtained by the photographic method.

Measurements in the infra-red.[2] Photographic and photoelectric methods can be applied to the near infra-red up to about 1μ, but beyond that there is no alternative to the thermopile or bolometer, of which the former is now almost exclusively used.[2]

The Müller thermopile (F 767) is a recent development with a very much greater sensitivity than the established types. It has been found in the Hilger laboratories to be about seven times as sensitive as the most sensitive other thermopile with which they are acquainted, and being enclosed it does not suffer much from unsteadiness.

The comparative slowness and lack of sensitiveness of thermopiles except in the red and infra-red render them barely worthy of consideration in the spectral regions where other devices are available, although it is possible to get useful results down to about 2500 A in the ultra-violet.

Observing fine-structure in absorption bands. The first essential is that the resolving power of the spectrograph should suffice for adequate separation of the bands. The method of measuring the intensity and position of fine structure lines is mentioned on pp. 47, 48, 88.

The use of a source of illumination having a line spectrum is ruled out, since important details of the fine-structure may fail to be revealed owing to

[1] A. C. Hardy, *J. Opt. Soc. Am.*, 18, 97, 165 (1929).

[2] For photographic plates for the infra-red, see p. 114.

insufficient lines in the spectrum of the illuminant. It may be said that if the fine-structure is finer than about 25-30 A a continuous source is best (see under " Continuous Light Sources " Chapter VII).

Obtaining absorption curves of highly photosensitive substances. For this kind of work a Notched Echelon Cell is very useful (see p. 75).

Summary.

The methods to be generally recommended in the characterisation of a substance by its absorption spectrum are summarised below :

Absolute Measurements of Extinction Coefficients in the Ultra-violet.

(1) Where the detail of the absorption spectrum (*e.g.* the separation of the peaks of absorption) is not less than 100 A at 2600 A [1]

Light source : tungsten steel spark (F 406).
Spectrograph : quartz, 20 cm. focus (E 370).
Photometer : the " Spekker " (H 290).

(2) Where the detail may be as fine as 30 A at 2600 A :

Light source : tungsten steel spark (F 406).
Spectrograph : quartz 60 cm. focus (E 316).
Photometer : the " Spekker " (H 237).

(3) Where the detail may be as fine as 10 A at 2600 A ;

Light source : Uranium electrodes (F 803).
Spectrograph : 170 cm. focus (E 383).
Photometer : the " Spekker " (H 382).

(4) Where, as in photochemical substances, it is important to obtain measurements with very short exposure, the notched echelon cell may be used (see p. 75).

(5) For the region beyond 1850 A the Vacuum Spectrophotometer with fluorite prism may be used (H 345).

Measurements of Extinction Coefficients in the Visible and Ultra-violet as far as Wave-length 2500 with the Highest Possible Sensitiveness.

(6) Photoelectric photometer (H 267) with ultra-violet Monochromator (D 33) or double monochromator (D 138). Light source : hydrogen tube (F 698). The measurement of absorption spectra having a number of bands by this method is very tedious ; it is particularly suitable where very small differences of absorption are in question. For the visible spectrum a Pointolite lamp is best.

[1] Or greater or less elsewhere in proportion to the varying dispersion of the instrument. The dispersion for various parts of the spectrum will be found in the Publisher's Catalogue E.

The absolute accuracy is limited by the means of adjusting the equality of the beams, but is somewhat superior to the apparatus 1, 2 and 3.

Recording Fine-structure.

(7) The method universally adopted is as follows :

Photographs are taken on a quartz, glass or grating spectrograph with hydrogen tube (F 698) the photograph then being put through a recording microphotometer.

Detail of 60 A, 30 A and 7 A (at 2600 A) will require spectrographs of 20 cm. (E 370), 60 cm. (E 316) and 170 cm. (E 383) focus respectively.

§ 3. THE CHARACTERIZATION OF A RADIATION, SPECTROPHOTOMETRY IN CONNEXION WITH COLOUR, AND THE COMPARISON OF LIGHT-SOURCES AND FILTERS,

The problems relating to the spectrophotometric comparison of light-sources, including of course sources provided with filters, are in some respects similar to the preceding ; but the ensuing paragraphs will serve to emphasize points that require special consideration in this type of measurement.

We are now interested in the ratio of the intensities of two radiations, instead of, as in the previous sections, the logarithm of this ratio ; and it is now $\Delta I/I$ that defines the sensitivity, where ΔI is the smallest detectable change in the light-intensity I. It should be repeated that within limits, $\Delta I/I$ for the eye and photographic plate is independent of I, whilst for the photo-cell, photronic cell or thermopile, ΔI is independent of I.

It is most often the case that the radiations to be measured are continuous spectra in which the rate of change of intensity with wave-length is not so great as it may be in absorption measurements in connexion with chemistry. Thus the considerations which in absorption work call for large dispersion (see p 47) do not often arise acutely in the work now in question.[1]

Fluctuations in brightness of a single light-source will not affect the true shape of the spectrophotometric curve appreciably if they are small ; if, however, a comparison between two different light-sources is in question, a curve being plotted wave-length by wave-length as with a visual or

[1] There is a kind of comparison of light-sources which is briefly dealt with later, namely, the comparison of line spectra, which forms the basis of spectrum analysis In this of course the dispersion must suffice to separate the lines of the metals which are sought.

photoelectric instrument, any change in intensity of one source during the measurement will result in a curve whose shape is misleading.

Thus it is desirable in such measurements to have the source considerably more steady than it need be in actual use. In the case of electric lamps this result must be achieved by using a very steady electric supply such as a battery, or, with similar sources of light it is sufficient to connect both to the same electric supply.

Application to Colorimetry.

No doubt it was with applications to colorimetry in mind that Guild so strongly disfavoured that class of instrument (which we will designate the "first type") in which "the observer views through the eyepiece of a spectroscope two adjacent spectra, one above the other, isolating a small wave-length range by means of adjustable shutters in the eyepiece," and favours a second type in which "the observer looks through a slit situated in the plane of the spectra .... and sees a suitably divided field"[1] of the dimensions generally accepted for photometric work.[2] The essential difference between the two types is that in the first the available light is concentrated into a slit image which constitutes the photometric field, while in the second type the available light is distributed over a relatively large photometric field.

It must be remembered that for large densities and with slits narrow enough to give a sufficiently pure spectrum for absorption spectrophotometry the amount of light available is very small, and the field of view will in no case be very bright. In each type mentioned there are two opposing features which respectively increase and decrease the accuracy of reading attainable. Thus the greater brightness of the field in the first type, as compared with that in the second, enhances the accuracy of readings in the measurement of high densities. On the other hand, for a constant brightness the standard field of the second type allows of more accurate reading than the narrow-slit field of the first type.

It is not obvious at first sight which of the two factors has the greatest effect and it is a matter of experiment to determine which of the two types will be better for a given kind of work.

A Hilger-Nutting spectrophotometer (an instrument of the first type) is easily converted into one of the second type by covering one-half of the object-glass of the spectrometer telescope with a prism of small angle, so that spectra normally closely adjacent become accurately superposed. On then removing the eyepiece and viewing the object-glass

[1] J. Guild, *Proc. Opt. Convention*, 1926, pp. 81, 82.

[2] A field of 2° has been recommended by Ives for flicker photometry and by Guild for colorimetry, *loc cit*, pp. 96, 132.

through the slit one sees the type of field used in the second class. In this way a very direct comparison of the two types was made. With various widths of the collimator slit and a telescope slit-width of 5 A at the D lines readings were made on a density of about 1·7. At a wave-length of 5890 A it was found possible for a skilled observer with great concentration [1] to take readings having a mean error of 0·03 in density with a collimator-slit opening of 4 A. By means of the slit field, however, it was possible to get readings with a mean error of less than half of this with a slightly smaller width of collimator slit (3 A at the D lines). Moreover it was possible to take these readings with far greater comfort, an important factor in taking a large number of readings.

Apart from this, in the second type there will be a tendency to obtain sufficient brightness by opening the telescope slit; and the use of an eyepiece to observe and limit this width according to the gradient of absorption is an additional adjustment and one liable to be overlooked in routine work by some of those who have to use spectrophotometers. Thus, as the result of experiment, it would seem that for accurate measurements of high density spectrophotometers using a slit field are not merely as good as the other type, but definitely preferable to it.

If, however, our interest is centred on the transmitted radiation rather than the nature of the absorbing material, the regions of less density become of paramount importance, and we may with advantage adopt the second type of spectrophotometer.

Photographed pairs of spectra examined visually or by microphotometer. As has already been seen, the probable error $\Delta I/I$ is independent of the exposure provided the plate be neither under nor over-exposed. To secure highest accuracy the most suitable type of plate is, as before, one with a large γ, with the plate recommended on p. 53 the limit of accuracy $\Delta I/I$ is equal to the limit of discernible density difference (observing the plate with the eye) 0·01 divided by $\log_{10}e$. That is, an intensity can be determined with this plate with an error of 0·01/0·43 or 2·3%. Photoelectric methods are far superior in accuracy for this work.

Application of the principle to quantitative analysis by emission spectra. It may be pointed out that in quantitative spectrum analysis by emission spectra, for a given method of exciting the spectrum of a given substance, the intensity of a line due to a metallic element is often approximately proportional to the percentage of the element present,[2] provided

[1] The difficulties of observing this field are threefold: (1) the very small brightness, (2) the very narrow eye-pupil necessitates keeping the eye very steady; (3) some diffusion of the image arises owing to the very narrow eye-pupil.

[2] F. Twyman and A Harvey, *J. Iron and Steel Inst*, No. 11, 397 (1932); F. Twyman and C S. Hitchen, *Proc R. S.*, A, **133**, 87 (1931).

that the line is not subject to reversal. One might therefore expect to obtain an accuracy of about 2·3% in the estimation of such an element. So far results of this accuracy have not been published though they have been attained in one or two laboratories. This may partly be due to limiting accuracy not having been sought, but we attribute it chiefly to the difficulty of running an arc or spark on two separate occasions in such a manner as to give radiations of identical character. Such a constancy would be strange ; it is not likely that the various metallic vapours in the arc or spark gap will be present in the same proportions on the different occasions, nor that the electrical conditions of excitation within the gap will remain constant. The selection of Gerlach's homologous line pairs [1] is a step towards avoiding the latter source of error.

Photoelectric measurements. It appears that for measurements of this character photoelectric methods are peculiarly appropriate, for in these ΔI is a constant so that by making I large we can make the error in measuring $\Delta I/I$ small.

Choice of a method for a particular purpose in this section. In comparison of light-sources which are liable to be unsteady a photographic method is preferable, so that exposure for the whole spectral range is made at the same time. We can then derive from the measurements a curve of comparison of the light-sources which is true at the time of taking the photograph. If comparison is made point by point, any individual measurements may be subject to a variation from the average value peculiar to the moment at which the reading was taken. If, however, a more sensitive photoelectric arrangement is available, it will be of advantage to use it provided that the variation of the source can be kept less than is detectable with the photographic plate.

The choice of a suitable method for other purposes in this section will depend on considerations similar to those already elaborated at the end of § 2, to which reference should be made.

[1] *Practice of Spectrum Analysis*, 6th Edition, p. 35, Adam Hilger, Ltd.

CHAPTER V

SOLVENTS

In Chapter II of Part I, attention was drawn to the fact that when a solution of an absorbing substance does not obey Beer's law, the cause is often to be sought in some interaction between solute and solvent. It is also well known that the maximum of absorption for the same solute often occurs at slightly different wave-lengths in different solvents, whilst cases have been observed where the character of the spectrum has completely changed. For instance, the absorption spectrum of acrolein dissolved in hexane contains three distinct maxima, whereas a solution in alcohol shows only a single wide band.[1] Effects of these kinds arise most readily when the molecules of the compounds concerned are "polar," *i.e.* have a permanent electric moment. It is clearly desirable, therefore, that in any investigation of absorption spectra the solvent selected should be non-polar or only very slightly so, particularly if the absorbing substance itself is likely to be polar. This condition is, of course, limited by the essential one that the solvent must be transparent over the whole range of wave-lengths to be covered by the observations, and also by considerations of solubility. For the earlier work on absorption spectroscopy, the solvents most commonly employed were water and alcohol, both of which are polar. More recently, non-polar liquids such as hexane, or heptane, and *cyclo*hexane have been used to a much greater extent, whilst for certain purposes, carbon tetrachloride and chloroform have been found to be suitable, although the latter is also slightly polar. Since these compounds as supplied commercially are liable to contain impurities which exhibit strong absorption bands (*e.g.* commercial cyclohexane contains benzene) whilst absolute dryness of the solvent is often essential, an account of methods of purification and drying which have been found to be satisfactory by experienced workers in this field of research is given below for a number of the compounds mentioned above, whilst the table shows the wave-lengths in A to which various thicknesses of materials so prepared are transparent.

These data were obtained with 1 min. exposures to a hydrogen lamp of medium power, taking only 300 milliamps at 2000 volts. With more

[1] Luthy, *Zeits. Phys. Chem.*, Vol. 107, p. 28, (1923).

intense sources, shorter wave-lengths are also transmitted. Thus, a 1 mm. thickness of the sample of cyclohexane actually transmits the 1854 A line of the aluminium spark spectrum.

TABLE II.

Solvent	1 mm	1 cm.	2 cm.	4 cm.
*Cyclo*hexane C_6H_{12} - - -	1900	1950	1990	2070
Hexane C_6H_{14} - - - -	1870	2010	2050	2090
Carbon Tetrachloride CCl_4 -	2450	2570	—	2620
Water H_2O - - - -	1870	1910	1930	1950
Ethyl Alcohol C_2H_5OH - -	1980	2040	2090	2140
Chloroform $CHCl_3$ - - -	2230	2370	2430	2460

Preparation of Pure Dry Solvents for Absorption Measurements.[1]

The details given in the following schemes for purification apply to initial materials of ordinary commercial purity. The quantities of the reagents specified are for 1 litre of solvent in each case. It is desirable to carry out distillations in an all-glass apparatus whenever practicable, particularly when transparency at very short wave-lengths is required.

Cyclohexane.

Probable absorbing impurities:—Benzene. Derivatives containing sulphur.

1. Shake with repeated charges of fresh oleum (up to 10% strength) until no further charring takes place.
2. Wash thoroughly with (*a*) distilled water, (*b*) weak alkali.
3. Shake for several hours with 5% potassium permanganate. Separate the *cyclo*hexane layer and wash out the permanganate with distilled water.
4. Stand the product over lime, and dry it by distillation from lime (three times) and from freshly-fused calcium chloride (three times).
5. The last traces of water can be removed by distillation from sodium, or by fractional freezing.

Constants. $d_4^{20} = 0{\cdot}7784$; B. Pt. 81·4° C.; F. Pt. 6·5° C.

[1] These methods are intended for the preparation of solvents of rigorous purity. For many purposes this is not essential, and the work can then be curtailed considerably, *e.g.* according to Dr. J. J. Fox, ethyl alcohol can be made sufficiently pure by allowing it to stand for 24 hours over stick potash, and refluxing for one hour. The alcohol is then distilled, the first and last 25% fractions being rejected.

Hexane.

Proceed as for *cyclo*hexane, completing the final drying by distillation from sodium.

Carbon Tetrachloride.

Probable absorbing impurity :—Chloroform.

1. Saturate the CCl_4 with chlorine and allow it to stand for two or three hours in bright light. This process should be repeated until the chlorine colour persists.
2. Remove excess chlorine by shaking with soda, and free the liquid from alkali by several washings with distilled water.
3. Remove water from the carbon tetrachloride by distilling twice from freshly-fused calcium chloride
4. Complete the drying by standing the distillate over P_2O_5 for about an hour (it is best not to leave the liquid in contact with the P_2O_5 for too long). *Decant the clear liquid* and distil.

Constants :—$d_4^{20} = 1{\cdot}595$; B. Pt. 76·8° C.

Chloroform.

Probable absorbing impurities :—Phosgene, chlorine. May contain free alcohol.

1. Shake with water for twenty-four hours, changing the water hourly.
2. Dry by refluxing over P_2O_5, and distil the product twice in an all-glass apparatus.
3. The chloroform should be stored in the dark and *redistilled as required*. It remains absolute for about twelve hours only.

Ethyl Alcohol.

Probable absorbing impurity :—Aldehydes

Distil from (1) a mixture of 20 c.c. water with 4 c.c. pure H_2SO_4.

(2) 10 gm. $AgNO_3$ with 1 gm. KOH

Dry by distillation from

(1) freshly-heated lime— three times.

(2) an Al : Hg couple—three times.

Redistil immediately before use.

The Al : Hg couple is prepared by immersing Al foil, previously cleaned with soda, in a saturated solution of mercuric chloride for about 1 minute.

Constants :—$d_4^{20} = 0{\cdot}7894$; M. Pt. −117·8° C. ; B. Pt. 78·5° C.

Methyl Alcohol.

Probable absorbing impurities :—acetone, methyl acetate, methyl oxalate, methyl formate, etc.

1. Distil from lime or soda.
2. Add anhydrous calcium chloride (700 gm.). The crystalline product formed ($CaCl_2 . 4CH_4O$) is filtered off and dried in vacuo. (Good crystallization is sometimes encouraged by refluxing the mixture on a water bath for two or three hours.)
3. Decompose the crystals by distillation with water.
4. Methyl oxalate and methyl formate can now be decomposed by distillation over KOH.
5. Dry the alcohol over anhydrous potassium carbonate.

Constants :—$d_4^{20} = 0{\cdot}7915$; B. Pt. 64·5° C.

CHAPTER VI

APPARATUS FOR ABSORPTION SPECTROGRAPHY IN THE ULTRA-VIOLET [1]

In this and the succeeding chapters dealing with specific instruments and techniques of absorption spectrophotometry, the aim will be to give an adequate description of one piece of apparatus suitable for each type of work, rather than to present a list of all the varieties of each type. All these will be of modern types in use to-day. Early apparatus and methods are described in Chapter III, or more fully in Kayser's *Handbuch der Spectroscopie*, Vol. 3, and Baly's *Spectroscopy*.

(a) Apparatus for Absorption Spectrophotometry in the Ultra-violet by Photography, the Quartz Spectrograph and "Spekker" Photometer.

(The manipulation of this kind of spectrophotometer is dealt with in Chapter VII.)

The first essential is a spectrograph and the type in use almost to the exclusion of all others is the fixed adjustment quartz spectrograph, the first of which was designed by one of the present writers in 1906. The latest form of these having a medium dispersion is the Hilger "all metal" medium quartz spectrograph (E 316). It records the whole spectrum from 2,000 to 10,000 A on a single plate, and has a wave-length scale which develops simultaneously with the photo-

[1] *The numbers which appear in the text refer to the Publisher's catalogue.*

graphs.[1] The optical material used is quartz,[2] the light being first collimated by a quartz lens, then passing through a quartz prism of the form due to Cornu which consists of two halves of right and left rotation (this to annul the double refraction of the quartz) and then through a lens system. In the latest instrument use is made of non-spherical lenses.[3] Although larger and smaller instruments are made (types E 1 and E 370), it is with this medium type that the bulk of modern absorption work in the ultra-violet has been done, and it generally suffices for all work which does not involve the resolution of fine-structure.

With such a spectrograph, or even with the Hilger small quartz spectrograph (E 370) (giving one third the dispersion), and with a suitable light source (see page 84) much valuable work has been done in the past. Even to-day such an outfit suffices for determining qualitatively the limits of transparencies of glasses, etc. But the time is long past when the spectrograph and light source could be considered all that is needed for work on absorption spectra. Means are required to measure the amount of absorption for each wave-length.

To satisfy this need Adam Hilger, Ltd. introduced about 1910 the Hilger sector photometer (H 16).[4] Immediately in front of the slit of the spectrograph is a bi-prism which receives the light from the source in the following manner. The light emanates from the source and may reach the slit by two alternative paths. An upper beam passes through a wedge prism. The beam then passes through a rotating sector, the aperture of which can be varied, and, falling on the bi-prism, is deviated by the lower half of that prism to pass axially along the collimator of the spectrograph. The second beam traverses first the absorbing liquid under examination, contained in a suitable cell, then through another wedge prism similar to the former one but so arranged as to divert the light upwards instead of downwards, and a rotating sector of fixed aperture. The beam is then diverted

[1] Or a scale in wave-numbers This is to be preferred for several reasons. First, the detail of an absorption curve is as a rule better distributed when plotted on a frequency scale. Secondly, the bands in fine structure are at equal frequency intervals. Thirdly, linear interpolation can be used over a longer range when a scale of frequencies is used

[2] Optical work of dense glass (E 332) can be substituted in order to obtain increased dispersion in the visible part of the spectrum

[3] Corrected by means of the Hilger Interferometers (British Patent 103,832).

[4] Also designed, like the later "Spekker" instrument, by one of the present authors. Howe, *Phys Rev.*, 8, 1916 (674-88), gives the first published description other than that in the Publisher's catalogue Earlier devices for the purpose by Pfluger (*Phys. Zeit*, 4, 861-2, 1903), Houston (*Roy. Soc., Edinburgh, Proc*, 31, 547-58, 1911) and V. Henri (*Phys Zeit*, 14, 515-16, 1913), did not come into general use

by the upper half of the bi-prism, and passes axially along the collimator of the spectrograph like the first. We thus have two beams fed into the spectrograph, the one capable of being varied in intensity at will by varying the aperture of the rotating sector, the other subject to the absorption of a known thickness of the liquid under examination. A series of photographs is taken on a single photographic plate with the variable sector set to different apertures. Each of these photographs consists of a pair of spectrum photographs in close juxtaposition, one of which is of reduced density throughout its whole length, the other—that which has passed through the material under test—being more dense than the first in certain

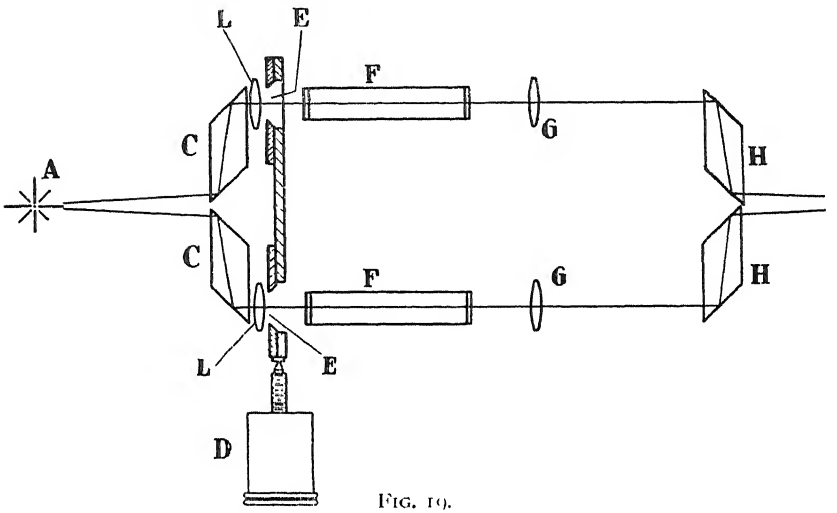


FIG. 19.

parts and less so in others, there being certain wave-lengths where the densities of the two are equal. The places of equal density of blackening being spotted, everything necessary is known for the plotting of the absorption curve.

This type of instrument has remained in use from its introduction to the present time, and much of the important absorption spectrophotometry by the photographic method has been done with it. It has been replaced by the "Spekker" Photometer[1] owing to the following disadvantages of the former instrument, which are avoided in the latter.

(1) Although investigations of the rotating sector form of photometer have repeatedly affirmed its accuracy within the conditions of experiment[2]

[1] Twyman, *Trans. Opt. Soc.*, 33, No 1 (1931-32).

[2] Twyman and Simeon, *Trans Opt. Soc.*, 31, 109 (1930) Doubts as to the validity of the rotating sector method were finally disposed of by the work of Brien O'Brien (*Phys. Rev.*, 33, 640, 37, 471; 41, 387); and of Webb (*Journ. Opt. Soc. Amer.*, 23, 157).

there is a possibility of fluctuations in the light-sources to a greater or less extent synchronising with the rotation of the sector, so that the radiation permitted to pass the sector is not purely a function of the aperture.

(2) Necessity of stopping the sector to change the adjustment, unless mechanism be introduced which is both complicated and also difficult to make accurately.

(3) Reduction of intensity of each beam to one-half by the sector disc.

(4) The difficulty of alignment. This is not due to the sector disc itself, but to the particular form of optical arrangement adopted for separating the two beams.

(5) Closeness of the scale at high densities.

The "Spekker" Photometer. The "Spekker" photometer is shown in a diagram in Fig. 19. It is placed in front of the spectrograph, such as a quartz spectrograph. Light from a suitable source of ultra-violet rays A passes towards the inner edges of two quartz rhombs C, C, from which beams are diverted upwards and downwards, to be then reflected forward through the tubes F, F, in one of which is placed the absorbing liquid, and in the other the non-absorbing liquid or whatever other substance may require to be compared—for instance, in the one tube may be an organic substance in a solvent, and in the other tube the solvent alone—the usual device for eliminating the reflection of the end plates. The beams from the rhombs pass one through a fixed rectangular aperture, and the other through an aperture which is variable by a micrometer screw, whose drum D is read by an index which travels along a helix in the drum. This not only simplifies readings, but acts as a stop to prevent the drum being turned too far in either direction

After passing through the respective absorbing and non-absorbing substances, the beams pass through lenses G, G of quartz, whose focal length is such that an image of the light source is formed on the face of the slit of the spectrograph. A second pair of quartz rhombs H, H are arranged to bring the beams of light together on to the slit in such a way that the image falling from the top rhomb and that from the bottom rhomb on the slit form a complete image of the light-source The beams through the slit pass on, without any vignetting in the interior of the spectrograph, to form images of the apertures at or near the prism, thence to be concentrated in the monochromatic images of the slit at the photographic plate. We may therefore be sure that parts of each spectrum line just above and just below the dividing line have intensities proportional to the areas of their respective apertures E, E.

The cell containing the absorbing liquid under test is placed in the upper beam, and a like cell containing the liquid with which it is to be

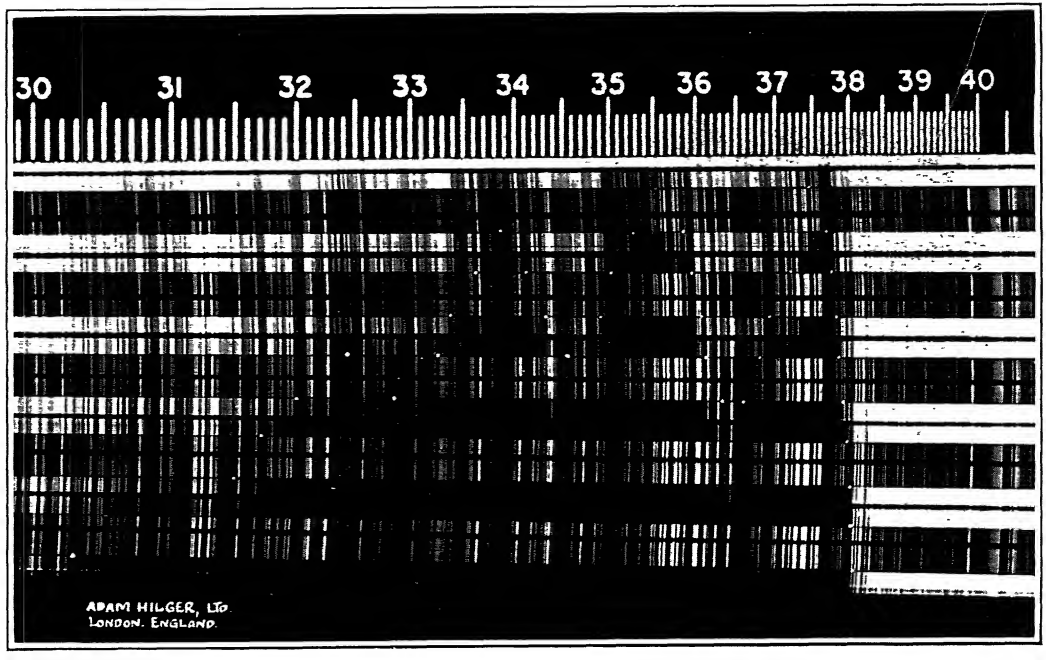


FIG. 20.

Enlarged portion of photograph showing absorption of anthracene in alcohol, obtained with Notched Echelon Cell outfit H 276, etc , and Hilger Quartz Spectrograph E 3. (For the corresponding curve, see Fig. 27).

PLATE X
To face page 72

PLATE XI

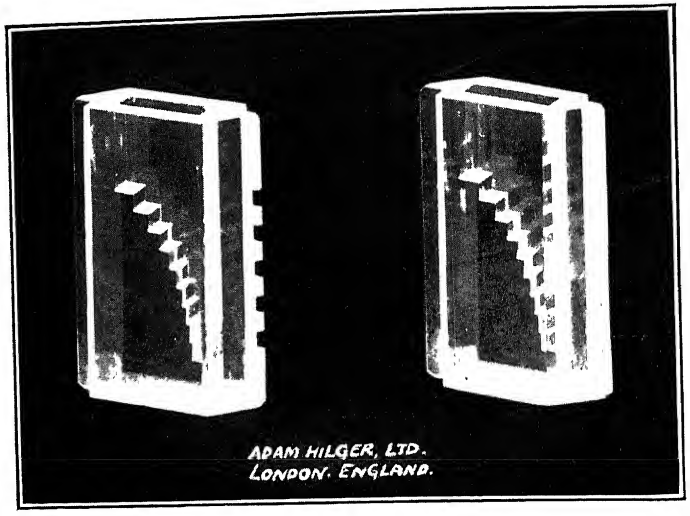


FIG 21.

Notched cell. Plain cell.

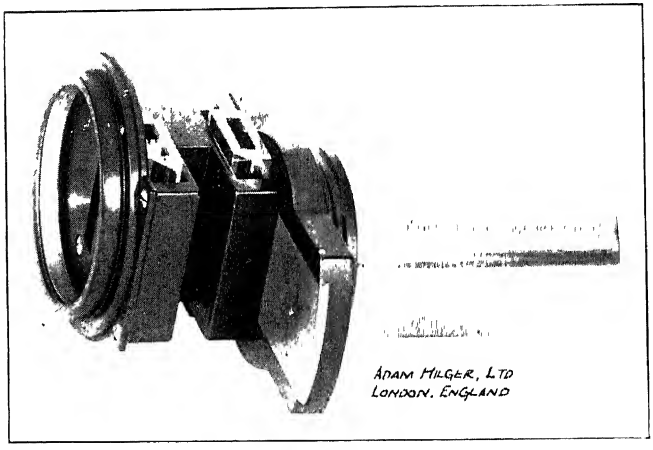


FIG 22

Cells in mount

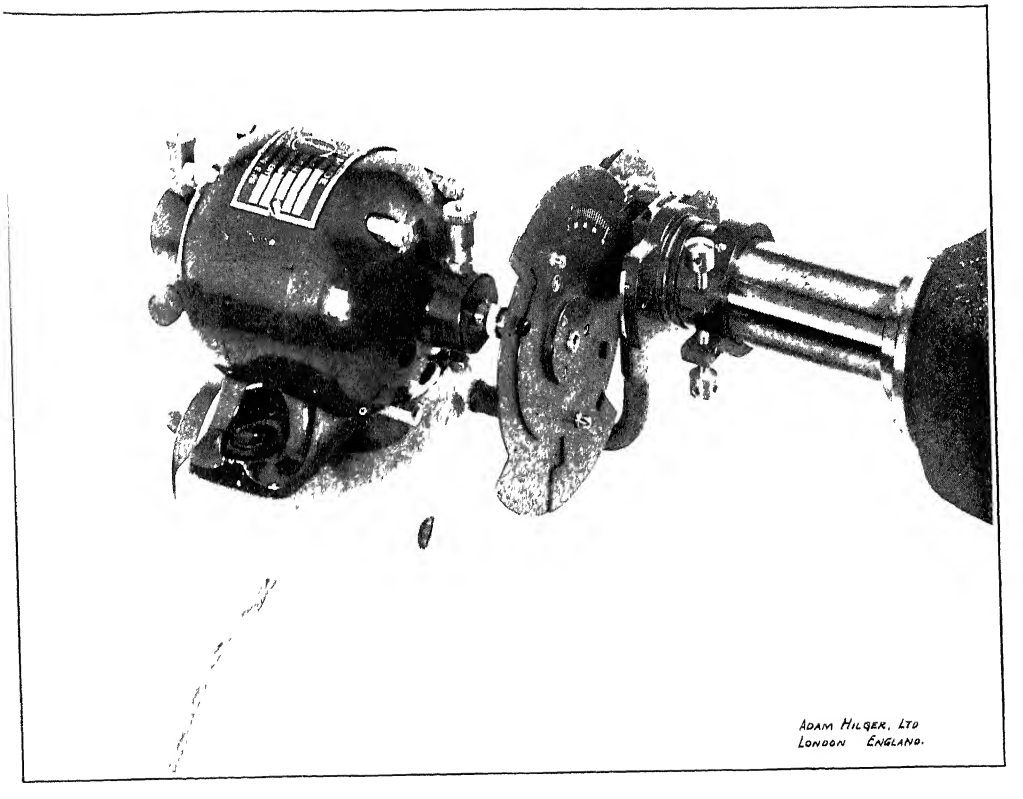


FIG. 23

Revolving Sector and complete Echelon Cell assembly on end of Spectrograph.

PLATE XIII

To face page 73

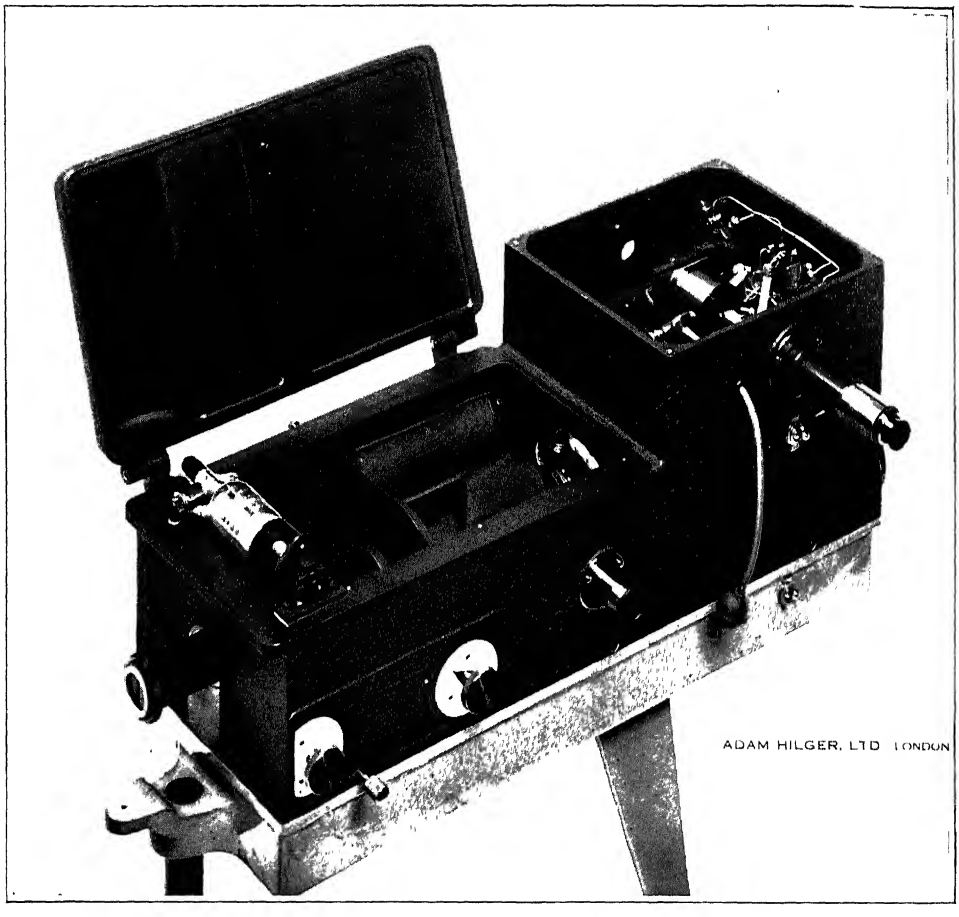


FIG. 24.

HILGER PHOTO-ELECTRIC PHOTOMETER

compared in the lower beam. If a series of photographs is then taken (see Fig. 17) as described in the case of the Sector Photometer, the positions of equal density of blackening can be spotted and their wave-lengths determined by reference to the wave-length scale.

The optical construction of the "Spekker" Photometer is such that at E and E there are equal fluxes of uniform radiation, which may be represented by I (per unit area). Let E_1 and E_2 be the areas of the apertures corresponding to the absorbing and comparison liquids respectively. Then the quantities of radiation (of a wave-length corresponding to a match-point) transmitted by the apertures will be E_1I and E_2I.

After transmission through the liquids these quantities become

$$(E_1I)10^{d_1} = (E_2I)10^{d_2},$$

where d_1 and d_2 are the densities of the absorbing and comparison liquids respectively;

whence $$10^{(d_1 - d_2)} = E_2/E_1,$$

or $$(d_1 - d_2) = \log (E_2/E_1).$$

The drum is engraved to read log (E_2/E_1) whence it is seen that *the readings give the density of the absorbing substance with reference to that of the comparison substance.* This fact stated in italics (which is not peculiar to this form of spectrophotometer) is not always borne in mind in recording absorption measurements.

This is a suitable place for considering why the comparison liquid cannot be omitted altogether so that the measurements actually give the densities of the absorbed substance. There are three reasons:

1. In a very large number of instances it is the comparative densities that are of interest, as in the case of a substance in a solvent, which solvent is used as the comparison liquid.
2. To use two cells annuls the effect of the reflections at the surfaces of the end plates of the cells
3. The effect of using a cell in one beam and not in another, or cells of different lengths in the two beams, is to alter the effective distance of the light source with resulting difference of intensity of illumination at the two apertures. There are instances where it is desired to obtain the total effective density of, for instance, a length of glass with plane parallel ends. In that case the glass can be used in one beam and nothing in the other, but the effect of the length of glass must then be calculated from data supplied by the makers of the photometer.[1]

[1] Should there be any reflecting surfaces in one beam and not in the other, the proportions of light reflected at each surface can be calculated from Fresnel's expression for normal incidence $\{(\mu - 1)/(\mu + 1)\}^2$.

Fig. 17 shows a portion of a photograph taken with the Spectrograph E 316 and "Spekker" ultra-violet photometer, and Fig. 25 the curve plotted from the data so provided. The wave-lengths are obtained by lining up from the spectrum line at which match occurs to the wave-length scales

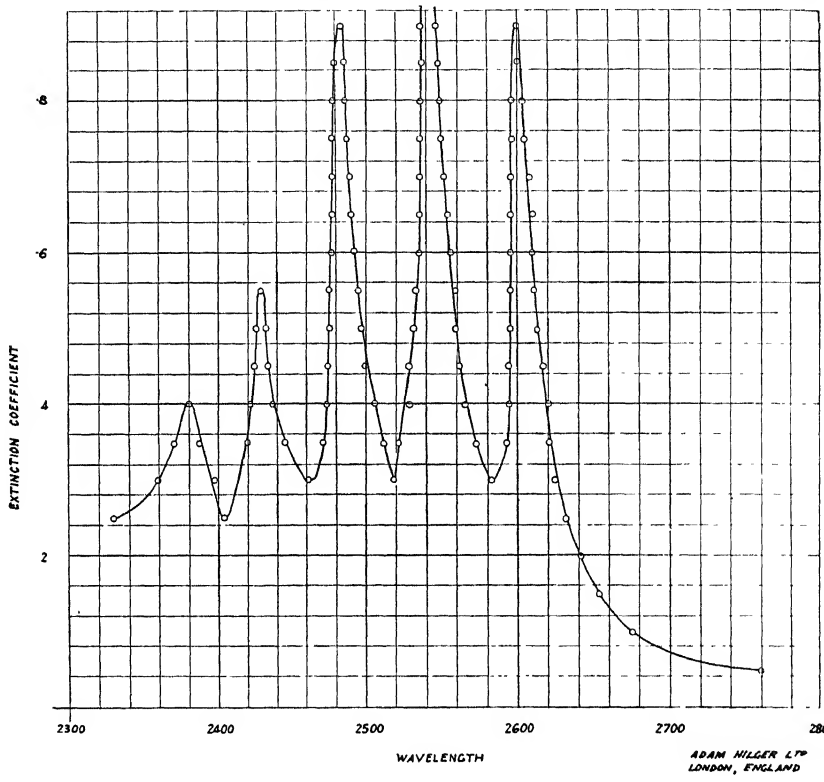


FIG. 25.

Absorption curve of benzene (in hexane). Measurements made on "Spekker" photometer used in conjunction with a Hilger E 316 quartz spectrograph: strength of solution 1 in 3300, length of tube 2·0 cm.; exposure 5 sec minimum, Wellington Anti-screen plate.

The photograph and curve shown were obtained with the "Spekker" photometer used in conjunction with a Hilger E 316 quartz spectrograph. See Fig. 17.

at the top and bottom of the plate; the extinction coefficients by dividing the corresponding reading on the photometer drum by the length of liquid in centimetres.

Smaller or larger spectrographs may be used in a like combination (see p. 70). If it is desired to carry the work into the visible region the quartz system of the spectrograph may, in order to increase the dispersion, be replaced with advantage by glass (E 332, etc.).

(b) Apparatus for Absorption Spectrophotometry in the Ultra-violet by Photography where great speed of Exposure is desirable. The Notched Echelon Cell.

Recent work in absorption spectrophotometry has shown that there is a great need for an instrument which will enable absorption curves to be obtained very much more rapidly than is possible with any of the photometers now in use. It is impossible with these latter to study any substance whose absorption is changing rapidly, and in many cases such changes are produced by the exposure to radiation which is necessary to obtain the absorption curve. The echelon cell has been introduced specifically for the study of such problems, but even in those cases where the absorption is not changing with time the instrument is of value in that it permits of a very great reduction in the time required to obtain absorption data, even when used for high extinction coefficients. An important feature of the instrument is that Hilger spectrographs (E 1, E 2, E 3, E 315, E 316, E 383, and the corresponding glass instruments) require no alteration whatsoever to permit of its use.

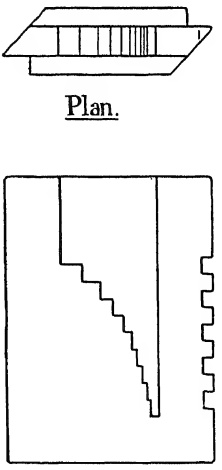


FIG. 26

Diagram of Notched Echelon Cell.

The principle of the method employed is given in a paper by Twyman, Spencer and Harvey,[1] and its more recent developments in a paper by Twyman.[2]

The appearance of the cells is shown in the photograph Fig. 21, and the details of construction are given in Fig. 26. Their external shape is that of a rhomb and the recess in which the liquid is placed has been formed in steps of diminishing length. The separate pieces of fused silica[3] forming the cells are adhesed so that each cell forms one solid piece without the use of any cement.[4] One edge of one cell is cut out after the manner of a comb whose teeth and spaces are of equal width. Owing to these teeth the spectrograph slit in front of which the cell is placed will receive light alternately from the notched cell and from a plain cell (exactly similar save only for the absence of the notches), placed in

[1] Twyman, Spencer and Harvey *Trans. Opt. Soc*, 33. (1931-32).

[2] F. Twyman *Proc. Phys. Soc*, 45, 1 (1933).

[3] Or crystalline quartz. At the date of publication of this description investigations are in progress for determining the most suitable material. Crystalline quartz cells are not adhesed.

[4] British Patent 103233/16.

parallel with it. The steps of the cells are so arranged that ten pairs of spectra, each consisting of one that has passed through the absorbing medium and one that has not, are photographed on the plate with the one exposure. In the manufacture of these cells it is claimed that an accuracy of 2% is obtained in the dimensions of the steps.

The cells are placed in a mount shown in the photographs, Figs. 22 and 23, with the notched one in the recess closest to the slit. The other recess is occupied by the plain cell. Between the two cells revolves the edge of a sector disc with variable openings. Light from the source (R) Fig. 27, passes through the condenser (C) and part of it enters the plain cell while part passes on to the notched cell. That transmitted by the plain cell which is filled with the solvent used for the solution, passes through the notches in the edge of the other cell after having been reduced in intensity in a known degree by the revolving sector disc, and thence to the spectrograph slit. That transmitted through the notched cell passes through the various thicknesses of solution and is reflected into the slit from the teeth of the notched edge. The resulting spectrogram (enlarged about three times) is shown in Fig 20.

The use of the plain cell containing the solvent automatically compensates for the losses by reflection at the various interfaces as well as for the characteristic absorption of the solvent itself.

Providing that the conditions are suitably chosen, it will be found that at one or more points in each pair of spectrograms the intensities will be equal. For those wave-lengths the density of the column of liquid is equal to the effective density of the sector. Then, if l be the thickness of the liquid and d the density, the extinction coefficient K is given by $K = d/l$.

A lengthy investigation has been devoted to determining the best shape of the cell. In the form that was finally selected as being the most suitable from every point of view, each thickness of liquid bears a constant ratio to the thickness of liquid next below it. The result of this is to make the changes in density for one step from the match point the same regardless of the height in the cell. Hence in terms of density, the accuracy of reading is constant and, since the density is constant for any one photograph, all the extinction coefficients will be known with the same percentage accuracy. Another consequence is that if, as preferred by some workers, the values of $\log_{10}K$ are plotted as ordinates then these values are equally spaced vertically on the absorption curve.

A great deal of care has also been devoted to the selection of the density range to be covered by the instrument and an examination has been made of a considerable number of the publications listed in *Recent Applications of Absorption Spectrophotometry*,[1] with a view to finding what are the

[1] Adam Hilger, Ltd.

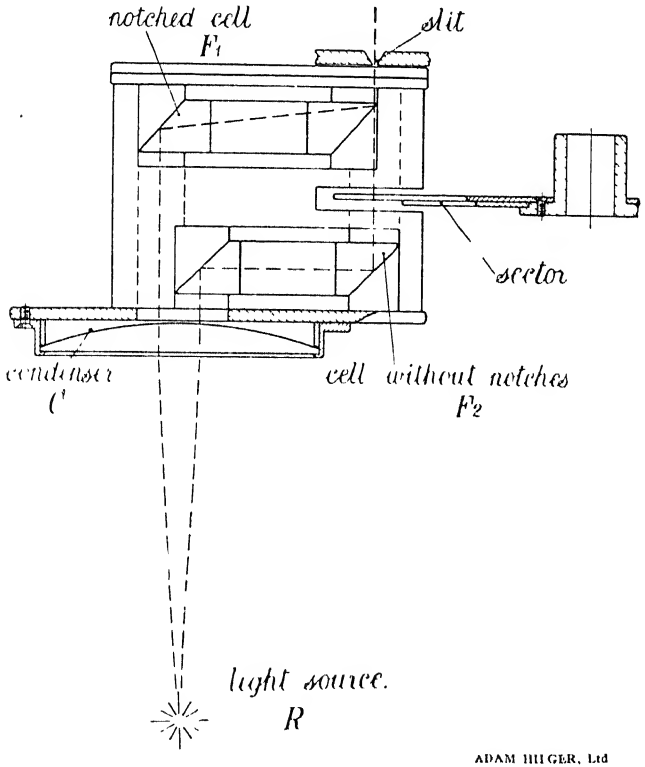
ranges of most use to workers in this field. By the provision of two rotating sectors the following arrangements are possible :

(*a*) Using the cell, which has steps of 0·1 to 1·0 cm., together with a sector giving densities from 0·155 to 0·398, permits extinction coefficients from 0·155 to 3·98 to be measured. This is regarded as the standard outfit and is supplied where not otherwise specified.

(*b*) Substituting a sector giving densities from 0·29 to 1·6, permits extinction coefficients from 0·29 to 16 to be measured.

FIG. 27.

Diagram of cells in mount, with sector.



It is believed that the range provided by these two combinations (*i.e.* from 0·155 to 16) is sufficient to meet every requirement

The mount for the echelon cells is provided with a slit which is alternative to that of the spectrograph The advantages of having the slit as an integral part of the echelon cell mount are as follows :

1. The slit is placed at such a position relative to the cell that the dividing lines are defined clearly on the photographic plate. The best position is not the same for all types of spectrograph, hence it is necessary that the spectrograph with which the echelon cell is to be used should be specified at the time of ordering the cell.
2. The slit is fixed by the makers in the correct position laterally relative to the cell, which is then correct once for all. The correct position of the light source is then easily found by inspection, as described in the instructions for use.

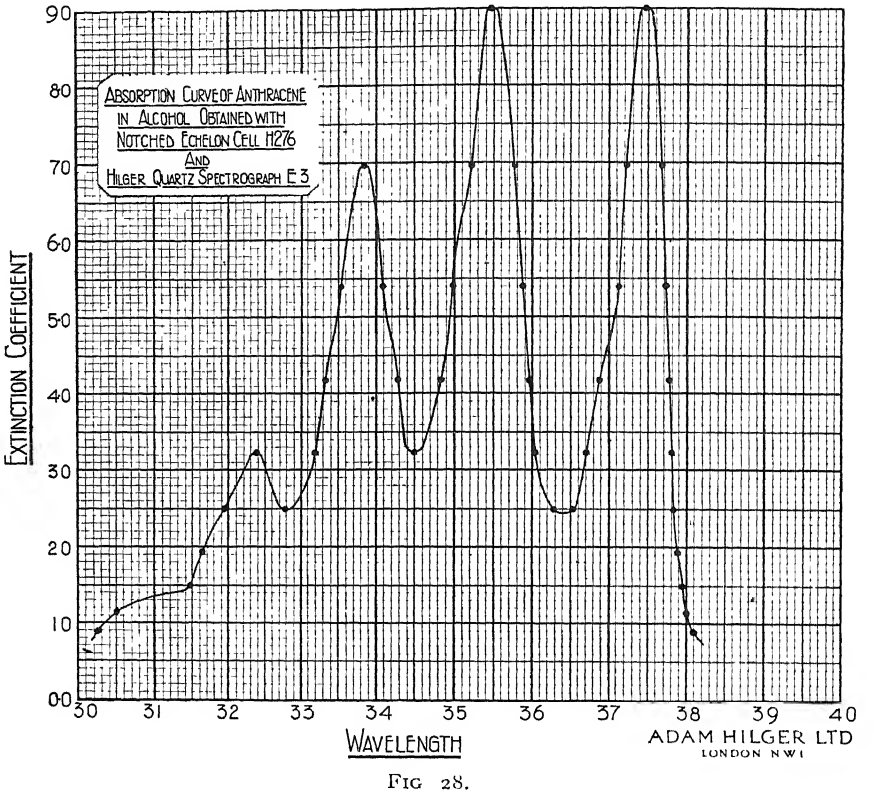


FIG 28.

3 As the cell is fixed relative to the slit there is no danger of its being displaced during an observation. It is readily placed in position when it is required to be used. This mount is provided with a quartz condensing lens which, when the light-source is set at a prescribed distance, ensures the correct conditions of illumination of the cells and the spectrograph.

The sector discs are carried on the shaft of an electric motor mounted

on a substantial tripod stand and provided with a locating device which ensures the sector disc taking up its correct position in the recess provided for it in the cell mount, without trouble. The driving gear on its stand is provided separately from the sectors which are readily interchangeable upon it. The complete assembly is shown in Fig. 23. The slit has fixed jaws which are customarily set to a separation of 0·03 mm.

RECORD SHEET—ECHELON CELL

Notes *Date*.....

$d =$

n	WAVELENGTHS	$1/l$	$K = d/l$
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Instructions d is the density of the sector as engraved thereon. The extinction coefficient is given by d/l, l being in cms. $1/l$ is given in the third column, and the extinction coefficient is obtained by multiplying the values of the third column by d.

FIG. 29.

In view of the great amount of information which is compressed into so small a space, difficulty may occasionally be experienced in reading the plates. To obviate this a reference plate can be supplied (H 296) on which numbers are photographed corresponding with each spectrum. The reference plate being placed on the negative and adjusted, the observer can see at a glance, whichever region of the spectrum he may be examining, the number of each spectrum strip.

Specially prepared sheets (Fig. 29) can be obtained for setting down the observations, and these are so arranged that the calculation of the extinction coefficients can be completed on the one sheet by simple multiplication

Comparison between the Hilger Rotating Sector Photometer, Notched Echelon Cell and "Spekker" Photometer.

It has been found, by visual examination of such photographs as those obtained in the above-mentioned instruments, that densities can be measured to within 0·02 to 0·04, independent of the absolute value of density.[1] Thus, in order that the percentage error in the measurement of the density shall be small, the density should be large.[2] The percentage error in the extinction coefficient is, of course, the same as that of the density measurement which is involved.

In the use of rotating sector photometers a large number of exposures are required, and a larger density, by increasing the time of each exposure will cause a serious increase in the time required to obtain an absorption curve. Further, the accuracy attainable in making such sectors does not justify the measurement thereby of densities greater than 1·5. In using the notched echelon cell, however, only one exposure is required; while the higher extinction coefficients, measured as they are with smaller thicknesses of liquid, do not involve the measurement of any higher density, and for these reasons it becomes feasible to measure large extinction coefficients.

To take an example, if in the use of an echelon cell of greatest length 1 cm., the density of liquid measured is 0·3,[3] giving extinction coefficients from 0·3 to 3 and requiring an exposure of about five seconds, an error may be obtained of from 7 to 13% in the measurement of the density and a like error in the values of the extinction coefficients.[4] If, however, the density of liquid measured is 1·5, giving us extinction coefficients from 1·5 to 15 and requiring an exposure of about 1¼ minutes, the error will not be more than 1½ to 3%.

The "Spekker" Photometer shares, though in a smaller degree, the fault ascribed above to the Revolving Sector type of photometer. As in the case of the latter instrument longer exposure is required at higher extinction-coefficients but, owing to its greater general rapidity, this is a less serious objection to the "Spekker" Photometer. Furthermore, the means adopted for adjusting the shutter system (a fine screw with which is associated a long helical scale on a drum) allows of accurate settings

[1] See von Halban and Eisenbrand, *Proc. Roy. Soc.*, A 116, 154. By suitable choice of photographic plates the accuracy can be increased, see p. 53.

[2] See Twyman, *Phys. Soc.*, 45, 1 (1933) and Chapter IV.

[3] Determined by the sector setting chosen.

[4] It is assumed that the cell is made with sufficient accuracy for the errors in thickness to be negligible, and this has been ascertained to be true in the cells which have been made.

being made for densities as high as 2. In both instruments a number of separate exposures are needed. With the "Spekker" time is saved between exposures which is lost in stopping and restarting the sector.

Thus it will be seen that the advantages of the Echelon Cell over both the "Spekker" Photometer and the Rotating Sector Photometer are

(*a*) Rapidity of measurement,

(*b*) Accuracy of measurement of high extinction coefficients.

The "Spekker" Photometer is, however, superior to the Echelon Cell in the measurement of very low densities, and has some slight advantage in the ease with which the plate may be interpreted owing to the more distinct separation of the pairs of spectra.

(c) Photoelectric Apparatus for Absorption Spectrophotometry in the Ultra-violet. The Hilger Photoelectric Spectrophotometer H. 67 with Müller-Hilger Double Monochromator D 138.

This is the combination which should be selected where the highest degree of spectral purity is essential, although for many purposes a single monochromator suffices (D 33).

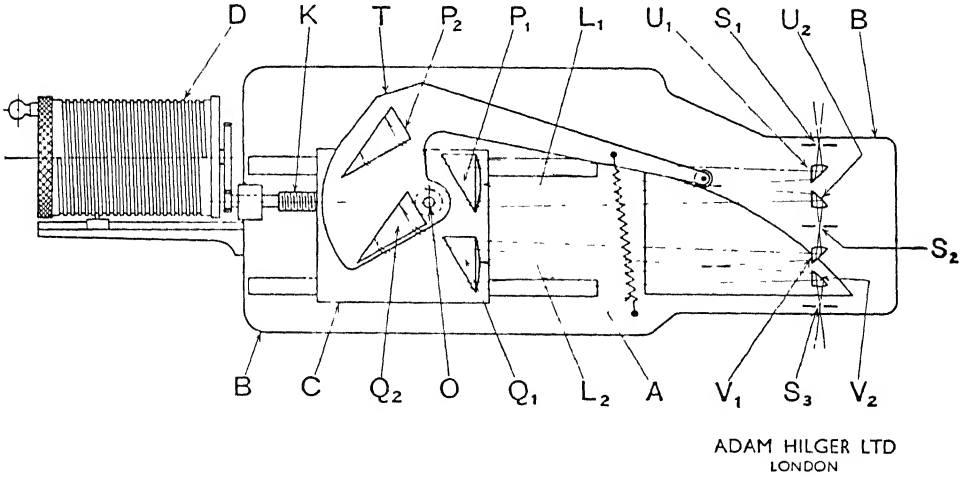


FIG. 30.

THE HILGER PHOTOELECTRIC SPECTROPHOTOMETER

The radiation is rendered monochromatic by a double Monochromator, Müller-Hilger type.[1] This instrument, the construction of which is made

[1] British patents, Nos 300786, 344722, 363264 and 374429.

clear in Fig. 30 (which illustrates the larger of the two models made, viz. D 107), consists essentially of two large aperture spectrometers in train on one base. Light enters at the slit S_1 and after traversing the prism system P_1 and P_2 forms a spectrum at slit S_2. This slit allows light of a certain required wave-length indicated by the wave-length drum to pass and traverse the second half of the instrument. Light of this wave-length, highly purified, emerges from the exit slit S_3.

Wave-length adjustment is made by a single hand control rotating the drum D_1 which reads from 0·185μ to 4·0μ for quartz. Accurate lens focussing for any required wave-length results automatically from the travel of the slide and the rotation of the prism table T.

The optical system is of quartz but is interchangeable with optical work of other materials such as glass or rock-salt. The dispersing prisms

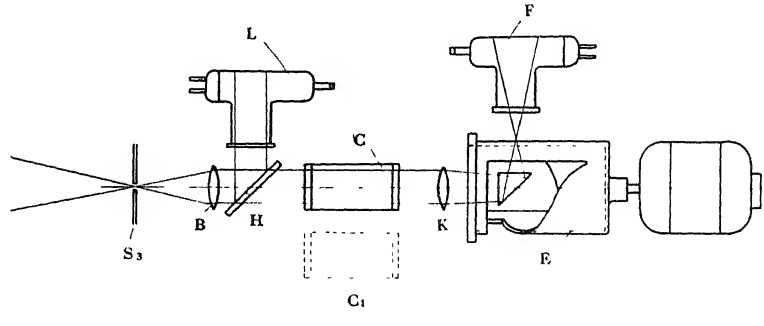


FIG. 31.

Optical System of Photo-Electric Photometer.

have an aperture of 60 mm. Aspherical lenses corrected by the Hilger Interferometer process are employed. The relative aperture varies from *f*/4 to *f*/5.8, the dispersion being approximately equal to that of four 60° prisms. Owing to the high dispersion, which enables wide slits to be used, and the large aperture, the instrument is of very high illuminating power. A smaller instrument (D 138) is also made and it is this smaller instrument which is generally used for this purpose.

The light emerging from the slit S of the monochromator enters the photometer (Fig. 31) and is rendered parallel by the lens B. The beam is divided into two parts by the quartz plate H. One portion is transmitted by this plate through the absorption cell C and is focused first by the lens K on to the rim of the variable cylindrical sector E, and then to the photocell F. Another portion is reflected by the quartz plate H into a second photocell L.

The rotating cylindrical sector E is shown in Fig. 32 as used, (*a*); and developed into a plane, (*b*). The light beam passes at right angles to the axis, and thus movement of the drum along the axis causes a change in the effective density. (This type of variable sector[1] which can be adjusted during rotation is much better than some other types in which

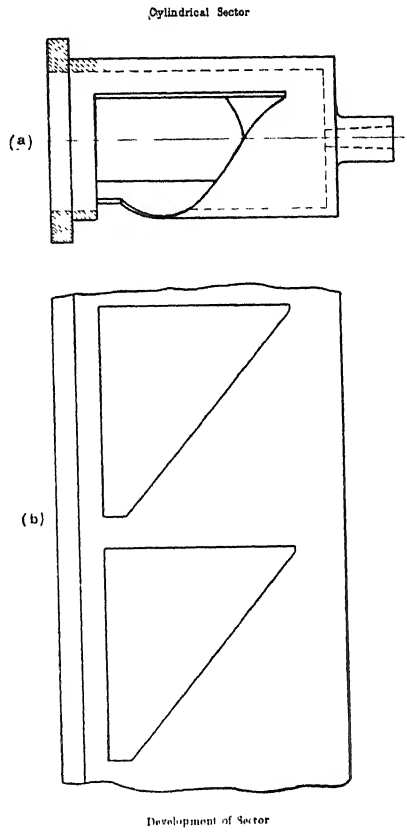


FIG. 32.

owing to wear of the various parts any calibration does not remain constant for long.) A scale is attached whose readings are easily converted into densities.

The two photocells have quartz windows, and are gas-filled. The currents from each are led in opposite directions to a Lindemann electrometer, so that only when the two currents are identical does the electrometer needle retain a steady position. This equality is roughly secured in

[1] Similar to that described by Dunn, *Rev. Sc. Instr.*, 2, 807 (1931).

the first place by adjusting the potentials in the gas-filled cells (keeping such a potential on each cell that adequate sensitivity is obtained), and thereafter by adjustment of the sector. The position of the sector is determined first with the absorption cell C in the beam, and then with the compensating cell C′, which usually contains the solvent. The instrument uses a "null method" and is thus independent of fluctuations of the light source. The complete photometer is shown in Fig. 24. The range of this photoelectric spectrophotometer depends on the photoelectric cell which is used. With a sodium cell it works from 5500 A in the visible to 2500 A in the ultra-violet. By the use of other cells the range can be extended from 1·2μ in the infra-red to the limit of transmission of quartz in the ultra-violet at 1850 A.

Of this photoelectric spectrophotometer it may be said that its sensitiveness ($\Delta I/I$) is at least ten times that of the best visual or photographic spectrophotometer.[1] The absolute accuracy of its measurements is limited by the accuracy of the sector. In an actual instrument this was within $\pm$ 0·2% of the aperture at 33% transmission (which corresponds with the optimum density for accurate measurement).

It appears then that this instrument can be relied on to measure densities in the neighbourhood of 0·43 to an accuracy of 0·004. This illustrates how instruments of the photoelectric type, indispensable for measuring small changes in extinction coefficients, do not show quite the same degree of superiority over visual or photographic instruments in absolute measurements of extinction coefficients. For measuring small densities (high transmissions) their superiority becomes unquestionable from any point of view.

CHAPTER VII

THE TECHNIQUE OF SPECTROPHOTOMETRY USING QUARTZ SPECTROGRAPH AND ULTRA-VIOLET PHOTOMETER[2]

Light Sources. (See also the general remarks in Chapter IV.)

There are three primary conditions for a light source for ultra-violet absorption spectrography (*a*) The source should be as free from fluctuation as possible ; (*b*) it should have a high intrinsic brightness ; (*c*) it should yield a spectrum as nearly continuous as possible.

[1] There is no difficulty in increasing this sensitivity von Halban and Eisenbrand have obtained a sensitivity 100 times as great as that of the photographic method.

[2] The numbers which appear in the text refer to the Publisher's catalogue

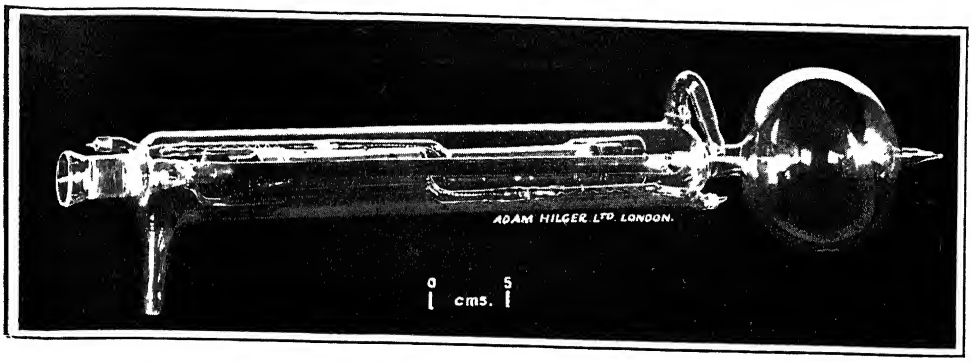


FIG. 33.

Hydrogen Discharge Tube (Bay and Steiner).

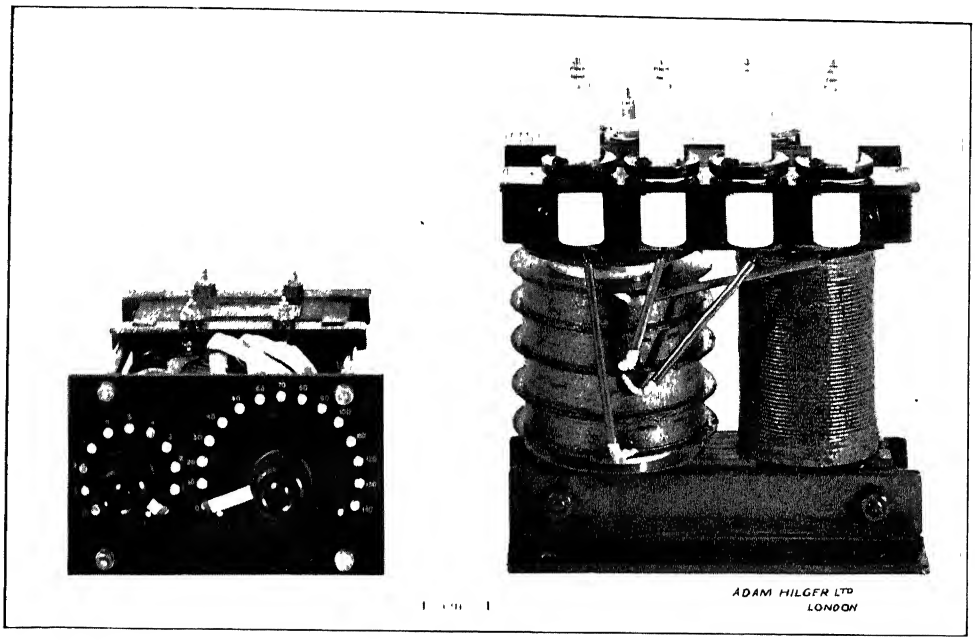


FIG. 34

2 K.V.A. Transformer Set.

PLATE XV

To face page 85

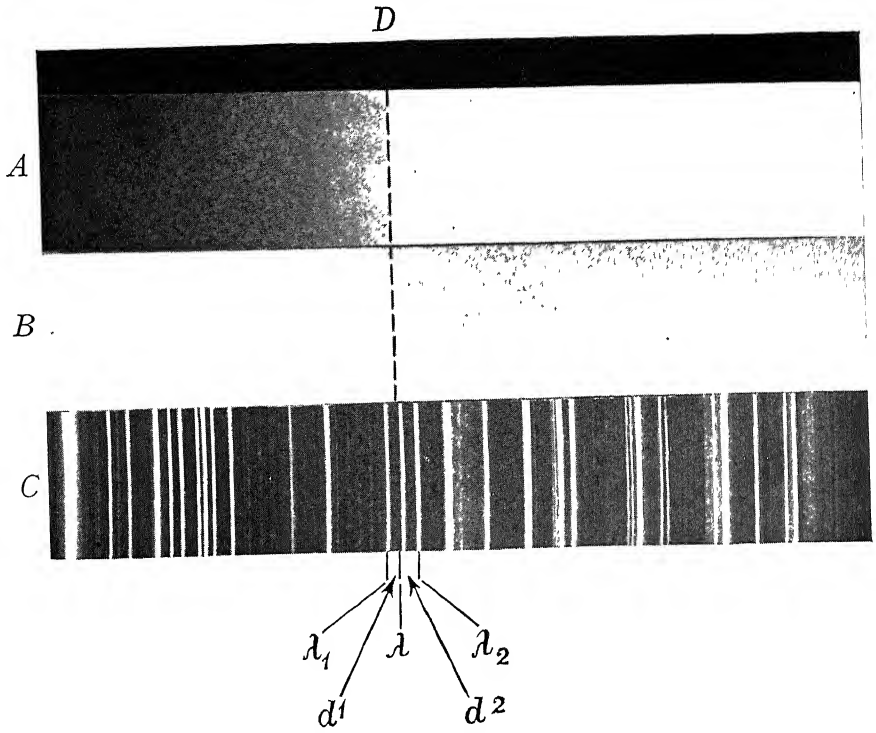
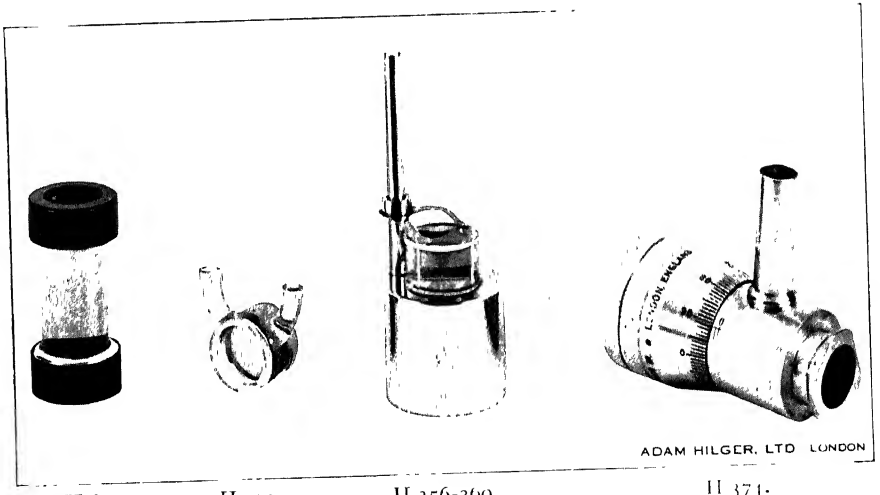


FIG. 35. Comparison Spectrum.



H 65. H 292. H 356-369. H 374.

FIG 36.—Cells for Liquids

Use has most frequently been made of a spark discharge between electrodes of a material which yields a very complex spectrum with a uniform distribution of lines. The spark is conveniently excited by a small ($\frac{1}{4}$ k.w.) transformer (F 282) fed with alternating current and giving on open circuit about 10,000 to 15,000 volts. A condenser (F 283) of sufficiently large capacity is connected in parallel with the spark gap. With a separation of the electrodes of about 4 mm. a brilliant "fat" spark is obtained.[1] The bright spark is accompanied by more noise than is comfortable, and a sound-proof box may be added with advantage (*e.g.* H 234). Sometimes, particularly with the aluminium spark (which, however, is not often used for absorption work) the spark will change its note to a hissing sound. When it is in this condition the radiating character of the spark is entirely altered and the radiation is much less intense. The brilliant character of the spark can be restored by scraping the electrodes.

The electrodes which are most suitable are of selected steel containing large percentages of tungsten and other metals. These yield an extremely complex spectrum extending to beyond 1850 A. They are obtainable (F 406) with one end sharpened to a wedge and when in use this edge should be placed so as to be collinear with the optical axis of the spectrophotometer. Then any wandering of the discharge will only take place along this axis and will have no ill effect upon the results. Such electrodes are consumed but slowly and may be used for several hours without readjustment.

With suitable precautions use can sometimes be made of an arc between electrodes of suitable material, such as the tungsten steel mentioned above. Disadvantages of the arc are the rapid consumption of the electrodes, rendering frequent readjustment necessary, and the large amount of wandering of the source of light, which is particularly objectionable when quantitative measurements of absorption are being made. This effect can sometimes be overcome by using a small area of a diffusing material as the actual source of light for the instrument. The diffusing medium may be a ground quartz plate over which is placed a metal plate pierced with an aperture about 4 mm. in diameter (H 191). The arc has the advantages of simplicity of operation and silence to offset partially its disadvantages. This diffusing plate is not recommended for use with the "Spekker" Photometer on account of the "granular" nature of the image on the slit.

Continuous Light Sources.

It has long been recognised that a light source giving a continuous spectrum in the ultra-violet would often be ideal for this work, whilst for

[1] For notes on the oscillating spark, see Appendix V.

detecting fine-structure such a source is indispensable. Various suggestions have been made and various types of apparatus employed for the purpose. The condensed electric spark under water has been employed by some investigators. It is very troublesome to operate and requires elaborate equipment including a Tesla transformer, while large quantities of distilled water are required for its operation.

Recently the Hydrogen Discharge Tube has been developed and forms a convenient continuous ultra-violet light source. The types of tube now generally employed are modifications of that first described by Bay and Steiner.[1] Such tubes are excited by means of transformers.

One improved form of the Bay and Steiner tube (F 698) is shown in Fig. 33. Connections are made to two wire loops which in turn are joined to the massive electrodes. The latter are surrounded by fused quartz tubes closed at the inner ends and opening to a long platinised discharge chamber near its extremities. The discharge is viewed " end-on " through a quartz window sealed to the main Pyrex tube by means of a graduated glass seal which is permanently vacuum tight. A reservoir bulb connected with the tube plays an important part in extending the life of the tube and maintaining its uniformity of operation. Cooling water circulates in the jacket to which connection is made through side tubes. To increase the working life of the tube it may be run at less than its full voltage, as for instance while setting up the apparatus with which it is associated. An important feature is that the discharge chamber extends to near the end of the tube, whilst the end of the tube is cone shaped In this way the radiation from the axial region of discharge reaches the spectrophotometer without any vignetting of the beam. The tube takes 250 m.A. at 4,000 v.

A suitable transformer outfit (F 700) for exciting the discharge is that illustrated (Fig. 34). It consists of an auto-transformer and a transformer and is designed for connection to 50-cycles A.C. mains. Rotary switches with stud contacts fitted to the auto-transformer permit of regulation of the output of the set The set illustrated is designed for an input of 150 volts A.C. and with both switches at the maximum position outputs of 4,000 volts or 2,000 volts can be obtained from the transformer depending on whether the two sections of the secondary are connected in parallel or in series. Lower outputs can be obtained by setting the switches to intermediate voltages. The full output of the transformer is 2 K.V.A.

There is one disadvantage against which the user of hydrogen tubes must be warned. The radiation comes from the interior of a tube, and if great care is not taken to maintain perfect alignment the radiation received by the two beams passing through the photometer may differ owing to the differing aspects from which the light source is viewed

[1] *Zeits. fur Physik*, 59, pp 48-52, 1929

through the two paths. Even so, unequal distribution of the illumination within the capillary may be a source of error. For this reason the condensed spark between uranium electrodes (F 803) may sometimes be used with advantage. The spark spectrum of uranium in the ultra-violet is very much richer in lines even than that of tungsten steel and has a noticeable amount of continuous background. The uranium metal is very expensive, but the electrodes may be clipped in holders which enable all but a minute portion of the metal to be used.

Determining Wave-lengths.

The E 316 spectrograph has a wave-length scale [1] so mounted internally that it may be printed on the plate and developed up with the spectrum. Wave-lengths are read from such a scale with sufficient

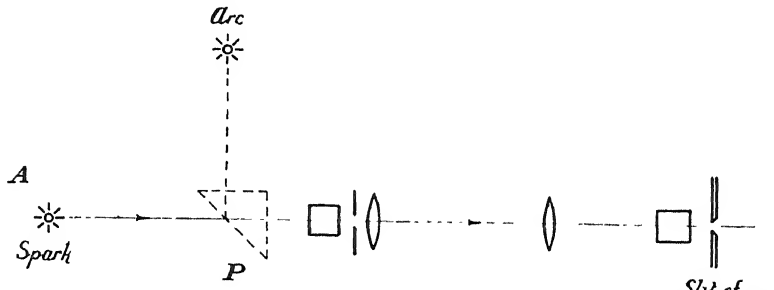


FIG. 37.

Method of Introducing Comparison Spectrum with "Spekker" Photometer.

accuracy where the reading of the match points is made by eye. Two kinds of work require something better.

(*a*) Where, as may sometimes arise, *e.g.* in the analysis of mixtures, the utmost accuracy is aimed at, the match points on the pairs of spectra obtained by the "Spekker" photometer may be determined by a photo-electric comparison microphotometer (II 364). It is useless to do this unless the wave-length of the match point is measured with a like accuracy. A method of doing this is to photograph on the plate, after each absorption exposure, and before moving the plate, an iron arc spectrum. Fig. 37 indicates one way of doing this. The diagram gives a plan view so that only the upper beam appears. A represents the beam passing through the "Spekker" photometer. The iron arc can be photographed by putting the prism P into the beam. When the exposure has been made it is only necessary to push the prism out of the beam, and the spark beam can then pass through the instrument uninterruptedly. In order that the iron arc

[1] Or a scale of wave numbers

may be impressed on the photographic plate without this being removed, and also without interfering with the pair of spectra, a special shutter must be added at the slit.

In the diagram, Fig. 35, A, B, are the absorption spectra, while C is the iron arc spectrum. Suppose the line D indicates the match point as shown by the microphotometer, and that this wave-length falls between two lines λ_1 and λ_2 in the iron arc. The microphotometer is fitted with a micrometer screw which enables the distances d_1 and d_2 between the match point λ and the wave-lengths λ_1 and λ_2 to be determined. The wave-length λ can then be found by linear interpolation if iron lines sufficiently close together are available or by using a Hartmann formula (see *The Practice of Spectrum Analysis*, 6th edition, pp. 55 *et seq.*). The lines of the iron arc spectrum are sufficiently close everywhere to allow the accuracy mentioned on page 56 of *The Practice of Spectrum Analysis* to be obtained on Hilger Quartz Spectrographs by linear interpolation, except in a few small ranges of the spectrum such as 2423 to 2438 A, 2211 to 2228 A, and 2115 to 2126 A. Greater accuracy may be secured by the use of the Hartmann formula although this adds considerably to the work as compared with linear interpolation.[1]

(*b*) In investigating fine structure in absorption bands, where the use of true spectrophotometers is impracticable, the wave-length or frequency differences between the lines, rather than the intensities, are required. These wave-lengths are usually determined with the help of a comparison (iron arc) spectrum, as has already been described above.

The Adjustment and Alignment of Ultra-Violet Spectrophotometers.

The remarks which follow are not intended to replace the full instructions for use of individual types of instrument but rather to indicate the most important general conditions to be observed in setting up such apparatus.

The light source, when separate from the photometer, is first set at the height of the middle of the spectrograph slit. It is then placed at the required distance from the slit and in line with the axis of the collimator. The plateholder is removed from the spectrograph and on looking into the extreme right hand side of the slot so revealed the observer will see the camera lens aperture. When the source is correctly in line a diffuse image of it will be seen in the centre of this aperture. The slit should be fairly wide and for final adjustment the length of the slit should be reduced to a few millimetres.

The "Spekker" photometers (H 237 and H 290) carry their own light source, and the adjustment is very simple, as it is only necessary to see that the light source is set mechanically in the prescribed manner,

[1] The Hartmann formula is given in *The Practice of Spectrum Analysis*, p. 56

the locating device on the spectrograph slit correctly engaged and the resultant diffuse image of the source properly centred on the aperture of the camera lens.

It is essential that the whole pencil of rays passing through the photometer should also pass through the lens system of the spectrograph. The instruments are designed to comply with this requirement when set up in the prescribed manner.

Selection of Exposures and Thickness and Physical Condition of the Liquid.

Exposure. The best results are obtained when the comparison spectrum (*i.e.* that which does not pass through the absorbing solution) has in each exposure a moderate degree of blackening, for those points in the spectrum where the match is made.[1] For this to be obtained the exposures should generally increase as the apertures are reduced. A satisfactory first result will usually be obtained by use of the formula :

$$\text{Exposure} = \text{A antilog } d$$

where A is a suitable exposure (which we may call the initial exposure) with the photometer at full opening, and d is the reading on the density scale of the photometer. A depends on the light source, the photographic plate employed, and the region of the spectrum where the point of match of the photograph in question is expected to lie.

With the sparking apparatus described above, tungsten steel electrodes, a spark gap of 3 mm., a slit width of six divisions on the drumhead (0·03 mm.), and Ilford Rapid Process Panchromatic plates, an initial exposure of 10 seconds with the Sector Photometer and one of 1 second with the "Spekker" Photometer usually gives a result of average utility.

The values of antilog d corresponding to the readings of the sector are given by the following table :

TABLE III

d	Antilog d	d	Antilog d
0	1	0.8	6.31
0.1	1.26	0.9	7.94
0.2	1.58	1.0	10.00
0.3	2.00	1.1	12.59
0.4	2.51	1.2	15.85
0.5	3.16	1.3	19.95
0.6	3.98	1.4	25.12
0.7	5.01	1.5	39.81

[1] See also page 64.

i.e. assuming that with the aperture fully opened, an initial exposure of 10 seconds is given, then with the aperture set at 0·1 one must make an exposure of $10 \times 1.26 = 12\frac{1}{2}$ seconds. The times so arrived at need only be complied with approximately.

It will be seen that the total exposure required for a set of sixteen photographs is about 150 times that of the first (full aperture exposure) amounting in the case of the Sector Photometer to about 25 minutes total exposure or, say 33 minutes counting the time of altering the setting of the photometer, shifting the photographic plate, and filling, cleaning and drying one cell. With the " Spekker " Photometer, requiring only about 1/10 the exposure this becomes 11 minutes.

With the sector type of instrument time can be saved in the exposure by using cells of various lengths, selected in such a way as appears below; using the Sector Photometer with a full aperture exposure of 10 seconds :

TABLE IV

Length of tube	Densities Measured	Extinction Coefficients Obtained	Total Duration of Exposure of the Group
2 cms.	0·2	0·1	
	0·4	0·2	
	0·6	0·3	240 seconds
	0·8	0·4	
	1·0	0·5	
1 cm.	0·6	0·6	
	0·7	0·7	
	0·8	0·8	330 seconds
	0·9	0·9	
	1·0	1·0	
0·5 cms.	0·55	1·1	
	0·6	1·2	
	0·65	1·3	about 225 seconds
	0·7	1·4	
	0·75	1·5	

Since the higher the density measured the greater the accuracy in measuring an extinction coefficient, it will be observed that the saving of time in exposures by using shorter tubes is accompanied by a lowering of accuracy in the determination of extinction coefficients.

The total duration of photographic exposure in the instance just given

is about 13 minutes. On the other hand three cells instead of one have to be filled, washed, cleaned and dried. We may take it then that the total time occupied is :—

Time of exposure - - - - - -	13 minutes
Altering the Photometer, shifting Plate for 15 exposures, say - - - - -	7 minutes
Filling three cells - - - - - -	4½ minutes

giving a total time of 24½ minutes as against 33 for the first example, a saving of 8 minutes approximately.

In the case of the " Spekker " Photometer the initial exposure required is only about one second and the total time for taking a complete series of photographs 2½ minutes. There is, therefore, no saving of time effected in taking photographs through different columns of liquid.

If, however, the extinction coefficients to be measured were higher, it would be found that the advantage of using different lengths of tubes is greater and one may sum up, therefore, by saying that having a variety of cells is of real utility in that it makes it possible

(*a*) to obtain, even with liquids of low extinction coefficient, a sufficiently high density to secure reasonable accuracy ;

(*b*) to avoid the exposures becoming excessive in the measurement of high extinction coefficients

It has been found that a series of cells covering all ordinary purposes, consists of the following :

1 mm , 2 mms , 3·333 mms , 5 mms., 10 mms , 20 mms , 40 mms., and 100 mms.

It will rarely be found worth while dealing with any one liquid in more than three cells.

It will not be amiss here to make some mention of the Notched Echelon Cell Using a density of 0·10 with an exposure of 1 1½ seconds, ten pairs of photographs extending over the same range as that given in the above tables are obtained. As pointed out elsewhere (see page 80) the Notched Echelon Cell is not so accurate for the measurement of low densities as the "Spekker" Photometer, in which long cells can be utilised.[1]

Concentration and Thickness of Liquid. The first step is to find such a thickness and concentration of the liquid under test as will give absorptions within the range of accurate measurement of the photometer.[2]

[1] In the standard instrument 10 cms , but instruments of this type are available taking much longer tubes

[2] As has been pointed out, Beer's law does not always hold, and where this is the case the observer must use the liquid in the concentration in which its properties are of interest to him

For this purpose set the scale to read 1.5. Then find by trial such a thickness and strength of liquid as will cause the spectrum photograph taken through the liquid to be slightly stronger in the region of maximum absorption than that taken through the variable aperture.

For liquids of very high extinction coefficient a micrometer tube (H 374) may be used in which thicknesses from 0·0 to 5·0 mms. may be readily obtained.

A convenient form of cell for use when very thin films of liquid are to be investigated, when their absorption is great or the available quantity is small, has been described by Judd Lewis. It consists of two quartz plates and a separating piece of thin tinfoil, clamped together by means of spring clips. The thickness of liquid can be determined as the difference between the sum of the thicknesses of the two plates and that of the assembled cell. Convenient sets of plates, in which pairs have identifying marks, thus avoiding repeated measurements, can be obtained (H 271).

To correct for the reflection of the end plates of the tube containing the liquid, the universal procedure is to use a tube in each beam, the one containing the solution under examination, the other the solvent only; or where the absorption of a pure liquid is being measured to use in the other beam some liquid of absorption negligible within the region under examination (*e.g.* distilled water).

Tubes for Liquids. A variety of tubes is available. They are illustrated in Fig. 36. The most generally useful (H 65) has screwed ends by which the quartz end plates are held firmly against the ends of the tube. H 292 is of fused silica in a single piece, the ends being attached by a special process without any cement or clamping. In H 356-360 the separating tube and end caps are held together in a spring mount, whilst H 374 is a micrometer cell in which the thickness can be varied from 5 mm down to a very thin layer, the thickness being read on the divided head to 0·01 mm.

The separating tubes or rings have polished ends, this being necessary in order to retain volatile liquids such as ether.

Both end plates and tubes should be carefully cleaned before putting them together. If all dust is removed from one end of the tube a plate may be gently slid into contact with it so that (if the tube ends are polished) a few broad coloured rings can be seen at the interface. The tube and the one end plate are then held together by the screw cap or other clamping device. The upper end of the tube is dusted and the solution is put in (by means of a pipette or otherwise) until a meniscus is formed above the end of the tube. If the carefully dusted end plate is now slid on quickly, firmly and steadily from one side, all air bubbles can usually be excluded

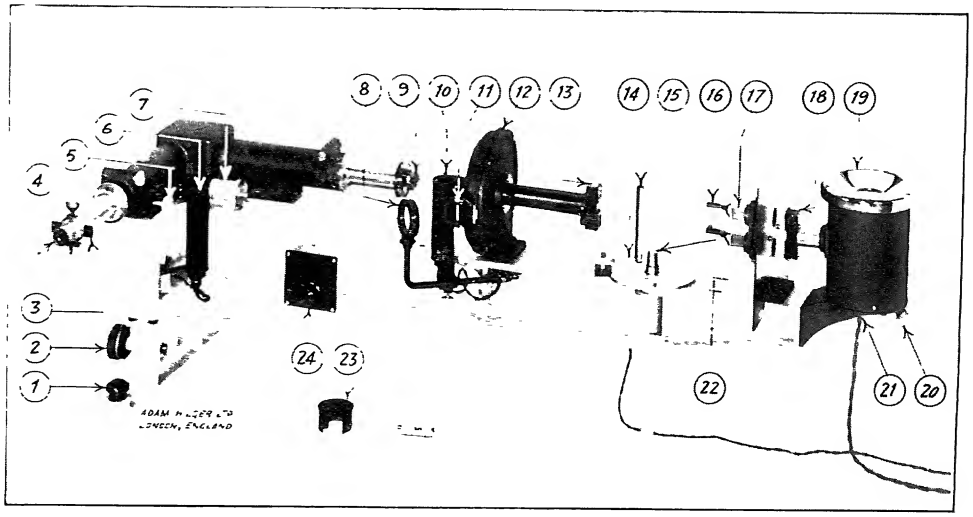


FIG. 38

Hilger Research and Industrial Spectrophotometer (see page 99).

PLATE XVI
To face page 92

PLATE XVII

To face page 93

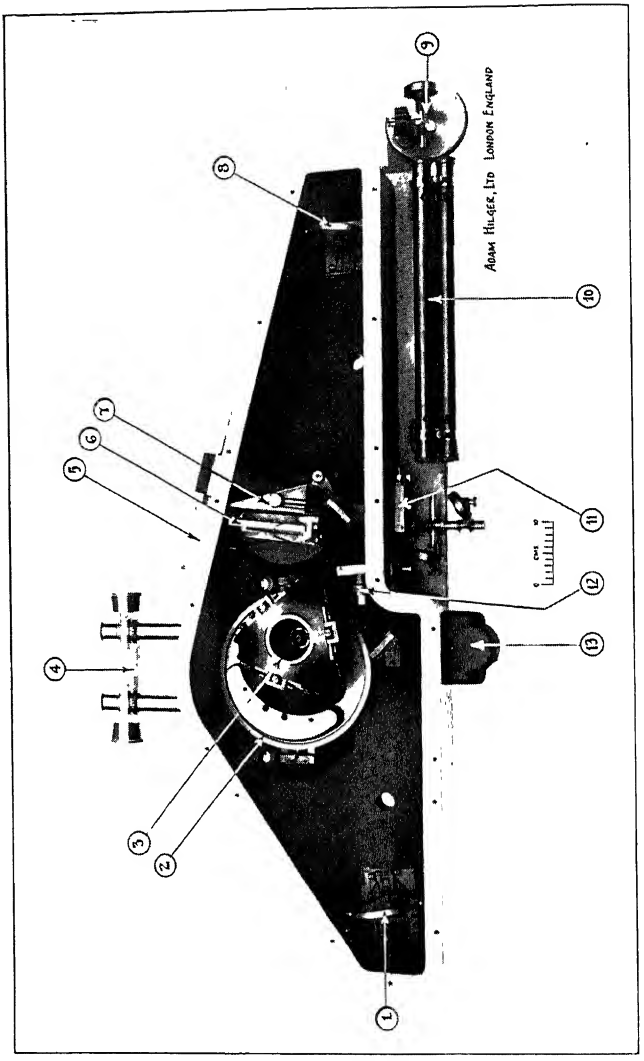


FIG 39

Prism-Grating Infra Red Spectrometer. Plan view with cover removed (see page 110).

and little or no liquid can get on to the outside of the end plate. It is better to start with clean end plates than to have to clean them when the tube is completely assembled. Any surplus liquid should be swabbed from the side of the tube. The upper end plate is now clamped and the filled tube is ready for use.

Great care should be taken not to scratch or chip either separating tube or end plates, or leakage will take place. Damage of this kind is most frequently caused by sliding or clamping the end plates in position with grit between them and the separating ring.

Taking the Photographs and Recording Results.

The method of taking the photographs has been briefly mentioned on page 71. A large number of photographs can be taken on the one plate since the space occupied by each double spectrum is rather less than three millimetres. A series of exposures should be taken with the scale set to a known series of densities, increasing the exposures in accordance with the table on page 89. Finally the wave-length scale of the spectrograph is printed on the plate as described in the Instructions for Use of the spectrograph.

If no wave-length scale is provided on the spectrograph, an arc should be used as comparison spectrum, in the way described on p. 87.

It is sometimes possible and permissible to omit some of the density settings from the series. Occasionally the nature of the absorption of a substance renders it of assistance to take two different thicknesses of liquid (for different regions of the spectrum) and to combine the results obtained with them. Two shortened series may then be taken on the one plate with a saving of time and material.

No instructions for developing and fixing the plate are required here, since the procedure is the same as in ordinary photography; a list of plates and developers having various characteristics appears in Appendix I, p. 113. The reader should also refer to p. 53 with regard to choice of plates.

When taking the photographs it is advisable to keep to a definite routine such as the following:

1. Set the plate carrier (and see that the shutter is withdrawn).
2. Set the photometer scale. (In the case of the Sector Photometer the motor can now be switched on and any notes made while it accelerates.)
3. Expose for the allotted time by switching on the light source.
4. Switch off the light source. (In the case of the Sector Photometer switch off the motor which will have come to rest by the time one is ready to repeat 2.)

Repeat the above in the same order for each density reading.

The room can be as well lighted as may be desired with this apparatus, the design being such that no special or irksome conditions of room darkening are required for its use.

The plate after washing and drying should be placed on a suitable viewing stand (F 644) and carefully surveyed with a low power eyepiece (H 245). Points are selected in each double spectrum at which the two spectra exhibit exactly equal degrees of blackening. It is a help in finding these positions if it is noted that the inequalities change over at a certain point from lighter at the top to lighter at the bottom. In each spectrum pair the position (or positions) of equality should be marked with a small dot. Fig. 17 shows a portion of such a negative obtained with a "Spekker" Photometer and an E 316 Spectrograph. The wave-length position is now to be determined either by reference to the scale or to the comparison spectrum.

Setting out readings. The readings so obtained are wave-lengths at which the absorption reaches a certain predetermined density. To convert the density readings to extinction coefficients, the values of the density are divided by the thickness of the liquid in centimetres.

Whatever region of the spectrum be in question, the best method of recording results is in the form of a curve which has either wave-lengths or frequencies[1] for abscissæ, and extinction coefficients or molecular extinction coefficients for ordinates. Occasionally the logarithm of the extinction coefficient is plotted.[2] Fig. 25 shows the absorption curve corresponding with Fig. 17.

The Examination of Translucent Bodies. It may happen that the materials to be measured are not perfectly transparent and cause a definite amount of diffusion of the light passing through them. This is the case, for instance, in the examination of skin or subcutaneous membrane for its transmission. It will be obvious in this connection that the effect of the diffusion is added to that of the absorption and erroneous measurements will result.

The question of diffusion was forced on the attention of those interested in measuring the density of photographic negatives, for the photographer is interested not in the quantity of light which in traversing the darkened part of a negative goes on in a straight line without deviation, but, since the printing paper is put in contact with the film side of the negative, he is interested in the total quantity of light that gets through. He is interested in measuring the light that is scattered as well as the light which is not

[1] Or more conveniently wave-numbers, see p. 6

[2] Not generally to be recommended, as logarithms of extinction coefficients cannot be added; but inevitable where a very wide range of extinction coefficients is being investigated.

scattered. The solution of the problem of correctly measuring the density in such a case was provided by M. Callier, the well-known photographic chemist.[1] The principle of his method is as follows :

If light from a point A is intercepted by a sheet of absorbing and diffusing medium B the intensity of light on an area C is diminished by the deviation of a certain amount of light in directions D, E, etc. In order that we may get an accurate measurement of the absorption we must supply light incident in all directions, and this was done by A. Callier by placing a sheet of diffusing medium on the illuminated side of B There

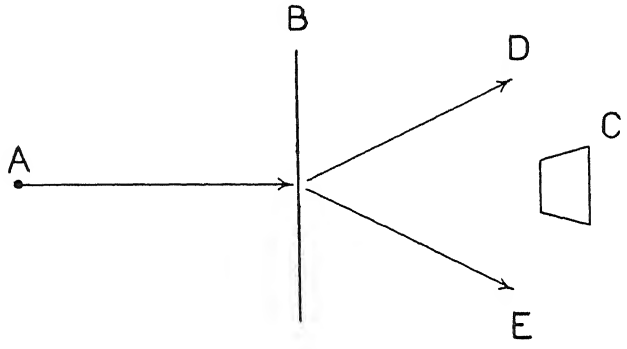


FIG. 40.

is difficulty in applying this to ultra-violet radiation because, for instance, if we select as the diffusing screen a plate of quartz with a frosted or greyed surface (and we have scarcely any other kind of diffusing medium to choose from) the diffusion is not perfect, but it has been found in practice by C. J. D. Gair that it is possible, with care, to obtain sufficient diffusion by using two such plates. Mr. Gair has in this way measured the transparency of human skin in the ultra-violet In this instance diffusion is of paramount importance.[2]

Conditions for securing accuracy in spectrophotometry. The reader will find this subject dealt with in Chapter IV.

The effect on absorption of heterogeneity in the solution. It is important that the absorbing medium be homogeneous If it is not, then the measured density will be smaller than that of a homogeneous solution

[1] "The absorption and scatter of photographic negatives," *Photographic Journ.*, 49, 200 (1909) Translated by C. E. K. Mees Callier distinguishes two densities, one for irradiation by diffuse and one by parallel radiation

[2] See also Pearson & Notts, *Brit. Journ. Radiology*, 6 (New Series), No. 86, August, 1933. Also Appendix IV.

of the same mean concentration, and the difference may be very large. The following examples may be mentioned.

When sulphuric acid and water are mixed the oily appearance due to incomplete mixing is very noticeable and very persistent. Blood, too, is so viscous that on dilution with water it does not easily mix, and special precautions are taken such as the use of glass beads in the dilution pipette. A similar effect has been found in the Hilger laboratory on diluting cod-liver oil with chloroform; when examined with the Hilger interferometer (a very powerful means of demonstrating heterogeneity in transparent media), the solution shows a granular appearance even after vigorous stirring, and this may persist for an hour or more.

In such instances as those given the effect may cause very large errors, especially when the absorbing substance has a high extinction coefficient.

The Effect of Temperature. Absorption sometimes varies considerably with temperature. Instances are given by Mukerji, Battacharji and Dahr.[1] In the former paper a case is recorded where a 30° rise of temperature caused a more than two-fold increase of density The 1933 Report of the National Physical Laboratory on p. 137 draws attention to the importance of temperature in measurements of absorption, and on p. 140 states that all such measurements at the Laboratory are made at or corrected to 20° C.

CHAPTER VIII

THE APPARATUS AND TECHNIQUE OF ABSORPTION SPECTROPHOTOMETRY IN THE VISIBLE SPECTRUM.[2]

OBSERVATIONS of absorption in the visible region were made very early; quantitative measurements of value commenced with Vierordt, of whose work mention has been made in Chapter II. Other important work that may be mentioned as of early date is Vogel's [3] determination of carbon monoxide in blood, and Auer v. Welsbach's separation of neodymium and praseodymium. Branby [4] described an absorption method for determining haemoglobin. His spectrophotometer was of the polarisation type using Nicol prisms, arranged so that the $\tan^2$ law applies.

[1] *Journ. Phys. Chem.*, 32 (1834), (1928); *ibid.* 35, 653 (1931).

[2] The numbers which appear in the text refer to the Publisher's Catalogue.

[3] *Ueber die Nachweisung von Kohlenoxydgas*, Berlin, *Ber. deut Chem Ges.*, 10, 1877, Jan—July, p 792.

Annales de Chimie, ser. V, vol. 27 (1882).

To-day there is no region of greater interest to the practical chemist. Not only are absorptions in the visible region particularly of interest because they are associated with colours and colour changes, as in solutions of dyes, fading, and so forth, but the apparatus is simple and speedy in use, and therefore suitable for the study of rapid changes in absorption, and of the nature and speed of reactions involving such changes.

The first requirement of the apparatus is a suitable light source, together with means of dividing the light from this source into two beams, of which one passes through the substance whose absorption is to be measured, while the other is either unabsorbed or passes through a comparison substance. The two beams are then passed through apparatus which can alter their intensity with respect to each other, and finally spectra of the two beams are formed in close juxtaposition by means of a spectrometer. The small wave-length range selected for an individual measurement is isolated, and the beams are brought to equality by the photometric part of the apparatus on which the reading is taken which gives the measure of the absorption.

All visual spectrophotometers consist essentially of the above elements. The instrument selected for description is the latest type of Hilger Research and Industrial Spectrophotometer (H 181). This has been designed with a view to chemical applications, and, before describing the instrument, it will be desirable to pass in review some typical instances of such applications of visual spectrophotometers, all of fairly recent date.

Typical examples of chemical applications of visual spectrophotometry. An accurate and rapid method of hydrogen ion concentration determination can readily be applied to plant control work. (Brode, *J A.C.S.*, 46, 581, 1924). In the dye industry, the determination of concentration and purity, since the absorption spectrum of a dye gives a measure both of its colour purity and concentration, can readily be made. Standard curves of pure commercially important dyes[1] have been prepared by the Dye Laboratory of the U.S. Bureau of Standards on a number of the most important dyes, Gibson et al., *Sci Papers Bur. Stds*, 18 (1922); Appel and Brode, *Ind. and Eng. Chem*, 16, 707, 1924; Appel, Brode and Welch, *Ind and Eng. Chem.*, 18, 627 (1926); Brode, *Ind and Eng Chem.*, 18, 708, 1926. In these papers data are given on the standard absorption curves for a number of dyes, methods for the spectrophotometric determination of the amount of dye present, detection of impurities, and in certain cases the quantitative estimation of the impurity present. In the quantitative estimation of dyes of known composition to determine the amount of salt or other non-absorbing material present, it is not necessary to determine the entire

[1] Including those permitted in foodstuffs

absorption band, but only the intensity of absorption of the maximum of the band. The ratio of this to the maximum absorption intensity of the standard curve gives the amount of dye present.

Other commercial applications include the absorption spectra of concentrated sugar solutions for determining the colour of sugar, Peters and Phelps, *Bureau of Stds. Tech. Papers*, No. 338 (1927), the study of coloured glasses used in photographic filters, for eye protection and similar purposes, Gibson, *J. Opt. Soc. America*, **13**, 267 (1926); *Sci. Papers. Bur. Stds.*, **18** (1922).

Reflection spectra measurements have been applied to the study of paint samples, paper samples, dyed fabrics and the like, to measure colour intensity, to compare samples and to obtain quantitative data on the rate and nature of fading of exposed samples, Gardner, *Physical and Chemical Examination of Paints, etc.* (1925), chap III. p. 34.

Other applications not so directly of interest to industry are spectrophotometric determinations of adsorptions, and in particular adsorption by biochemical materials, Fodor and Riwlin, *J.C S.*, I. 102 (1926); Fodor and Mayer, *Kolloid Z.*, **40**, 41 (1926); and Riwlin, *J.C.S.*, II, 2300 (1926). The dissociation constants of indicators can be accurately determined by a spectrophotometric examination of the indicator in solutions of known hydrogen ion concentration, Brode, *J.A.C S.*, **46**, 581, (1924); Thiel and Wülfken, *Z. anorg. Chem*, **136**, 393 (1924); Mellon and Martin, *J. Phy. Chem.*, **31**, 161 (1927). By the use of the same principle of observation a rapid and accurate method for the determination of hydrogen ion concentration has been developed. Although the method is colorimetric in principle it may be applied to coloured solutions as well as to colourless solutions.

An important field of research has been opened by the study of the effects of physical conditions on absorption spectra of various materials. The following are examples of recent researches of this type: the influence of heat on the absorption spectra of coloured alkali halides, Phipps and Brode, *J. Phys. Chem.*, **30**, 507 (1926), Flechsig, *Z Physik*, **36**, 605 (1926); studies of Beer's and Kundt's laws, Rossi and Basini, *Annali. Chim., Appl.* **16**, 299, 1926; Vaillant, *Compt. rend*, **184**, 1059 (1927), Szilárd *Bio. Chem. Z.*, **170**, 185 (1926).

Independent investigators searching for element No 61 (Illinium), used visual absorption spectra measurements in determining the cutting points in separating fractions of rare earth salts, Harris, Yntema and Hopkins, *J.A.C S.*, **48**, 1585, 1594 and 1598 (1926); Rolla and Fernandes, *Z. anorg. Chem.*, **157**, 371 (1926). The absorption spectra of various elements and salts in the vapour state have been the subject of a number of recent investigations, Sowerby and Barratt, *Proc. Roy Soc. A.*, **110**, 190

(1926); Zumstein, *Phys. Rev.*, **26**, 765 (1925); Van Heel, *Proc. K. Akad Wetensch*, Amsterdam, **30**, 95, 1927.

A method has been proposed for the spectrophotometric identification and determination of nitrates and nitrites by their colour reactions, Tassilly and Savoire, *Compt rend.*, **183**, 887 (1926). Similar applications have been made to the analysis of cobalt complexes of organic amines, Uspenski and Techibisov, *Trans. Inst Pure Chem. Reagents*, Moscow, p. 32 (1927), and to the constitution of ferro and cupri nitric oxide salts and ferro nitro sulphide compounds, *Ber.* **59 B**, 406, 411 (1926).

Choice of Design.

It will be seen that most of the above examples fall under the heading "The characterisation of a substance by its absorption" in Chapter IV, and, as is there shown, such applications require an instrument which will measure high densities with accuracy at wave-lengths precisely selected and known.

The Industrial and Research Spectrophotometer has been developed, with these desiderata in mind, from the earlier Hilger-Nutting Spectrophotometer It embodies a novel polarizing system produced as the result of research first undertaken in 1928. This system has a number of advantages, the most important of which is that the density scale follows the law $I = \frac{1}{2} \log \tan \theta$ instead of $I = 2 \log \tan \theta$, resulting in a more open scale at higher densities The theory of the system has been fully described by J H Dowell,[1] but it may be briefly stated here that the earlier system consisting of a fixed "polarizer" and a revolving "analyser" has been supplemented by a second fixed "analyser." The additional analyser has the additional effect of cutting out almost all the stray scattered light which leads to inaccuracies of measurement which are of special importance at the higher densities and when very intense sources (such as may be requisite for very strongly absorbing materials) are employed. The system has the further advantage that the emerging light has its plane of polarization constant in direction, so that no variation in intensity can take place such as might be caused by reflection from the surface of the dispersing prism or other optical parts as is the case when the plane of polarization of the emerging light rotates.

Fig 38 shows the general arrangement of the spectrophotometer. The light source (a pointolite lamp) is contained in the housing (19) and the light from it is separated into two parallel beams by the device (18) They then pass through the tubes containing the liquid under observation and enter the photometer (13) by two windows In the photometer the two

[1] Dowell, *J. Sci. Instr.*, 8, 1931 (382).

beams are brought into closely adjacent paths and subjected to the relative variations in intensity brought about by the polarizing prism system. A lens system focusses their line of separation on the slit of the spectrometer portion of the apparatus. When observed in the eyepiece the field of view is divided into two horizontal strips separated by an almost invisible line. Rotation of the photometer circle (and hence the movable analyser prism) causes variations in their relative intensities enabling compensation to be made for the absorption of a substance.

The rotating circle (12) is engraved with angular and density scales $d = \log I_1/I_2 = 4 \log \tan \theta$ and is totally enclosed; it is operated by the large milled head (2) underneath the spectrometer telescope, and adjacent to this is a switch (1) for the illuminators (6 and 11). The brightness of these lamps (6 and 11) can be varied by the control (24). The telescope is focussed by the screw (5). It will thus be seen that all the controls are conveniently grouped together, and the scales are easily legible to the observer without his having to move from the observing position. The slit (9) is symmetrical.

The eyepiece fitting is provided with a pointer which may be turned to one side out of the field, and with symmetrically opening shutters to restrict the range of spectrum under observation, the opening being read by small illuminated notches spaced 0·02 in. apart and located above the centre of the field of view.

As described above, illumination is by means of a 100 c.p. "Pointolite" lamp. For certain kinds of work, however, a Sphere Illuminator may be preferred, and can be supplied in place of, or in addition to, the "Pointolite" lamp.

The sphere is about 27 cm internal diameter and is illuminated by two 400-watt projector type lamps. A mount is provided at the back of the sphere on which a specimen can be attached in one beam, and a magnesium-coated surface in the other beam. The mount is reversible so that the specimen and magnesium surface can be changed from one beam to the other. For testing material (*e.g.* fabrics) with a pronounced structure shields can be attached in front of the lamps so as to screen out direct illumination.

When transmission tests are to be made, the above mount is interchanged with another having a magnesium-coated surface in the centre, a pair of deflectors being added in front of the sphere to obtain two equally illuminated beams. The complete unit is self-contained and can be interchanged with the pointolite lamp in a few moments.

Attachments are available for testing fabrics or reflecting surfaces. When using the fabric holder the lamphouse arm is rotated to 90° about

the axis (22) the 100 c.p. lamp being replaced by a 500 c.p. "Pointolite" lamp. The tube-holder mount (16) is removed and the fabric holder mounted on a fitting (22) which is an extension of the spindle on which the lamphouse rotates. A pair of auxiliary lenses is attached to the front

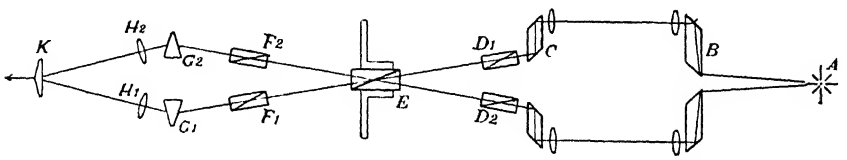


FIG. 41.

of the photometer box (13) by means of a milled head. An index attached to the fabric holder enables the angle to which it is rotated to be read off the scale (14), and hence the angle at which the light is incident on the substance can be determined.

The attachment for measuring the reflecting power of polished surfaces consists of a platform, to be mounted on the rod (15), carrying a prism and two accurately parallel surfaces. Each specimen is placed with its reflecting face in contact with one parallel surface and the prism so adjusted as to reflect the light on to one of the surfaces from which it is reflected on to the other and back again as many times as desired, finally entering the upper aperture of the photometer. Thus the absorption of the surfaces can be increased to any desired extent until it is readily measurable. The prism may be set in one position for a large number of reflections and can be moved for a less number of reflections. The comparison beam is reflected into the lower photometer aperture by a comparison prism underneath the platform.

Selection of Thickness and Concentration of Solution.

The thickness of solution to be selected depends on the nature of the solution.

In investigations on seven dyes permitted in foodstuffs carried out at the Bureau of Standards, Washington,[1] the investigators used cells of 2 cm. length, as did Walter C. Holmes [2] This is a very suitable length for general work in the visible spectrum.

A good general principle to govern the selection of tube length and concentration is to aim that the spectral regions of greatest interest (usually the peaks of the absorption curves) should be measured in the most

[1] Described in Scientific paper, No 440, June, 1922, "The spectral transmissive properties of dyes"

[2] *Industrial & Engineering Chemistry*, August, 1923 (acid dyes of the patent blue type), and January, 1925 (basic fuchsins).

accurate part of the scale of densities.[1] The extent to which this can be carried out will depend on the material available and the closeness with which Beer's law is followed.

As in the case of ultra-violet measurements it may be found of advantage to use two or more thicknesses of medium and combine the results from them when the curves have maxima of very widely differing densities.

Taking Measurements and Recording Results.

The substance whose absorption is to be measured is interposed in the lower beam (if it is a tube of solution it is usual to place in the upper beam a " control " of a similar tube with either water or the solvent in it). The tubes should be filled in the manner described on page 92. Except that they are provided with glass end plates they correspond exactly with those used for the ultra-violet photometers, except in the case of the 100 mm. size, which has a central cup. In this latter case both ends are carefully cleaned and put in place and the solution poured into the central cup, all bubbles being removed in the process. The shutters in the eye-piece are set to contain a range of spectrum which should not be great enough to show any variation in brightness or hue; hence it will depend on the steepness of the edges of the absorption bands to be investigated and on the part of the spectrum under observation. The slit should be of such a width (say 1-4 drum divisions) that the purity of the spectrum is not marred, while sufficient light is admitted to the field of view.

On rotating the circle the two portions of the field of view will be seen to alter in their relative intensities until they match exactly, and this position being attained the circle reading is noted.

Readings can be taken at any desired wave-length interval (read from the spectrometer drum) throughout the portion of the spectrum showing absorption, the closeness of the intervals being regulated by the steepness of the absorption curve. The correctness of the wave-length setting should occasionally be checked in the manner described in the instructions for use.

A few workers prefer to set the density scale to chosen readings, and to rotate the wave-length drum until a part of the spectrum is found at which equality of both fields occurs. It is claimed that this is quicker and less tiring than the former method

It is essential, if the most accurate results are to be obtained, that so far as possible the observer should be comfortable and the room properly darkened. The observer's eyes need to get used to the comparatively dim illumination of the field of view, especially at high densities, and it is important that the scales from which readings are taken should not be more brightly illuminated than is absolutely essential, if loss of sensitivity

[1] Viz. where $\Delta d/d$ is least, see p 57.

is to be avoided; hence the provision of the dimming switch. Physical discomfort and bad ventilation of the photometer room are found to militate against the best and most consistent results.

When taking readings a number of settings should be observed recorded and averaged. In general experience it is found that a number of quickly taken readings give a more accurate average than a few readings taken with long periods of viewing.

Interpretation of the Angular and "Density" Scales of the Hilger Nutting Photometer. If a reading d on the "density" scale be obtained with a thickness l centimetres of liquid or other absorbing substance, then the extinction coefficient is d/l.

If desired, readings may be taken on the angular scale. The density scale is derived from the angular scale by the use of the formula:

$$d = 4 \log_{10} \tan 0$$

where 0 = reading given by angle scale.

The Spectrophotometric Comparison of Light Sources. The Measurement of the Relative Spectral Distribution of Light Sources is discussed in *The Measurement of the Spectral Distribution of Light Sources by means of the Hilger Nutting Spectrophotometer.*[1] It does not come within the purview of this book.

The Measurement of Translucent Substances. As in the case of ultra-violet investigations of translucent media (see page 94) a diffusing screen should be interposed in the path of light in contact with the absorbing medium whose density is to be measured.

Use of the photographic method with the above instrument. By adding one of the Hilger standard cameras absorptions may be measured by the photographic method. Kodak I plates are suitable for this purpose.

CHAPTER IX

THE APPARATUS AND TECHNIQUE OF ABSORPTION SPECTROMETRY IN THE INFRA-RED, AND THE MEASUREMENT OF THE RAMAN EFFECT

PHOTOGRAPHIC plates specially sensitized for the red and near infra-red, now make it possible to take absorption spectrograms up to 12,000 A[2] but beyond this it is necessary to use a thermopile. No photoelectric or

[1] Adam Hilger, Ltd.

[2] See "Photographic Plates for use in Spectroscopy and Astronomy," Eastman Kodak Company (Obtainable from Adam Hilger, Ltd.). Infra-red Plates are also made by Ilford Ltd. and Agfa

selenium cells are sensitive in the infra-red beyond a wave-length of about 1·5μ.[1]

Work in the infra-red was in 1909 carried on by only a very limited number of workers, who had elaborated their own pieces of apparatus.

Before designing an infra-red instrument suitable for the general scientific worker, one of the present writers visited Prof. Paschen, then at Tübingen, whose advice seemed likely to be the most valuable of any to be had, and would like to take this opportunity of acknowledging the helpful advice given on that occasion.

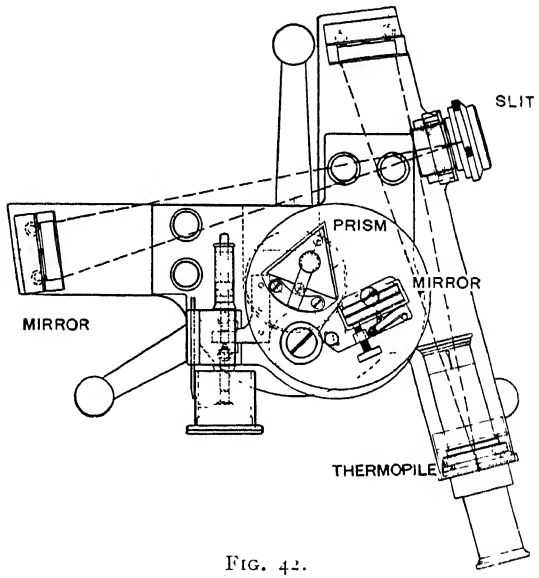


FIG. 42.

It was Prof. Paschen's measurements of refractive indices in the infra-red of quartz, rock salt, fluorspar, and sylvine that provided the instrument maker with the means of designing an instrument, reading in wave-lengths direct, which could be used by the chemist for investigating the emissions and absorptions which are characteristic of molecules.

The result was the infra-red spectrometer (D 83 etc.) of which a plan is shown in Fig. 42, and of which a general view is shown in Fig. 43. Light, after passing through a slit, is in this instance collimated by a mirror. It passes through the prism, is reflected by a plane mirror to a second concave mirror which forms an image of the spectrum on a second slit, behind which, in a well-protected metal casing, is situated

[1] It is claimed that a certain new cell is infra-red sensitive, but the claim appears to be founded on the ability to detect radiations transmitted through ebonite. Ebonite, however, is transmissive at 1μ.

the thermopile. The radiation falling on this produces an E.M.F. which is measured on a sensitive galvanometer (F 441). The prism and mirror are rotated by a micrometer screw of exactly the same kind as that used in the well-known constant deviation wave-length spectrometer, the drum head of the screw having just the same helical slot in which the index runs, reading in wave-lengths direct.

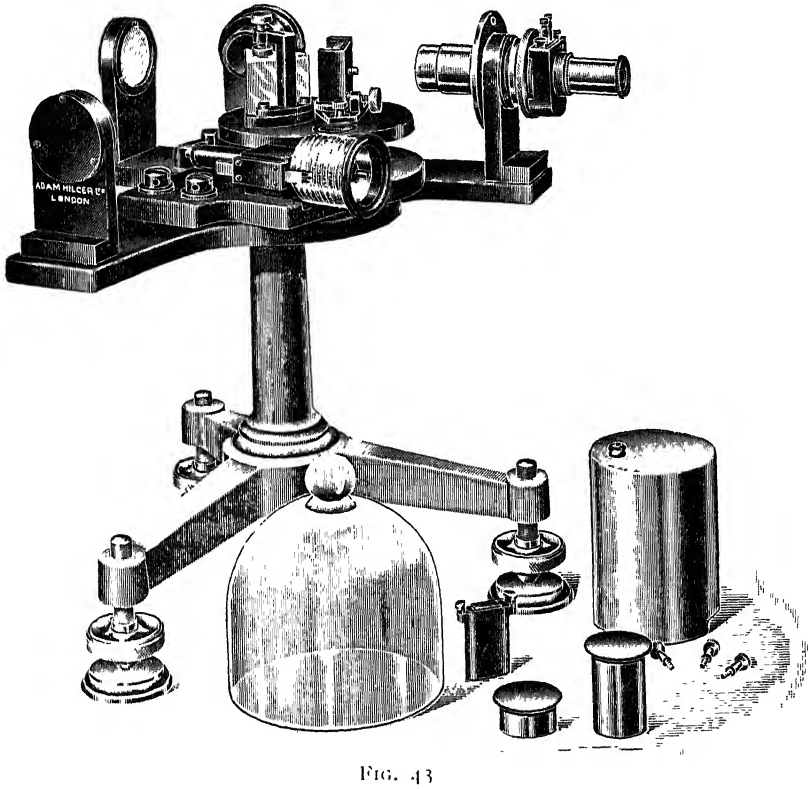


FIG. 43

The arrangement of prism and mirror, in which every ray when in the position of observation passes through the prism at minimum deviation, was first described by Wadsworth[1] as applied to a visual spectrometer. The arrangement used on the infra-red spectrometer differs, however, from that of Wadsworth, in that the mirror is differently orientated relative to the 60° prism.

In order that various ranges of spectrum can be examined with the greatest accuracy prisms are now available of the following materials: quartz, rock salt, sylvine, and fluorite.

[1] *Phil. Mag.* (5), 1894, **28**, 346.

The ranges of wave-length for which the calibration is made are as follows :

Prism.	*Range of calibration.*
Quartz.	0.185 μ to 3.5 μ
Fluorite.	0.38 μ to 9 μ
Rock salt.	0.38 μ to 16 μ[1]
Sylvine.	0.38 μ to 21 μ

From the diagram given at the bottom of this page a choice can be made of the best material to use for any given research, having regard to the dispersion and the range of spectrum transmitted. In the diagram the full lines represent the range of calibration for the various materials. The lengths of the lines represent the relative dispersions.

The process of plotting infra-red absorption spectra consists in taking measurements with a sensitive galvanometer of the electromotive force developed by the thermopile in the various portions of the spectrum (*a*) with the absorbing substance in, (*b*) with the absorbing substance out, or a comparison substance substituted, (*c*) with the radiation entirely cut off. Deflections of the galvanometer are closely proportional to the radiation falling on the thermopile, and one therefore obtains in this way three readings from which the extinction coefficient can be found.

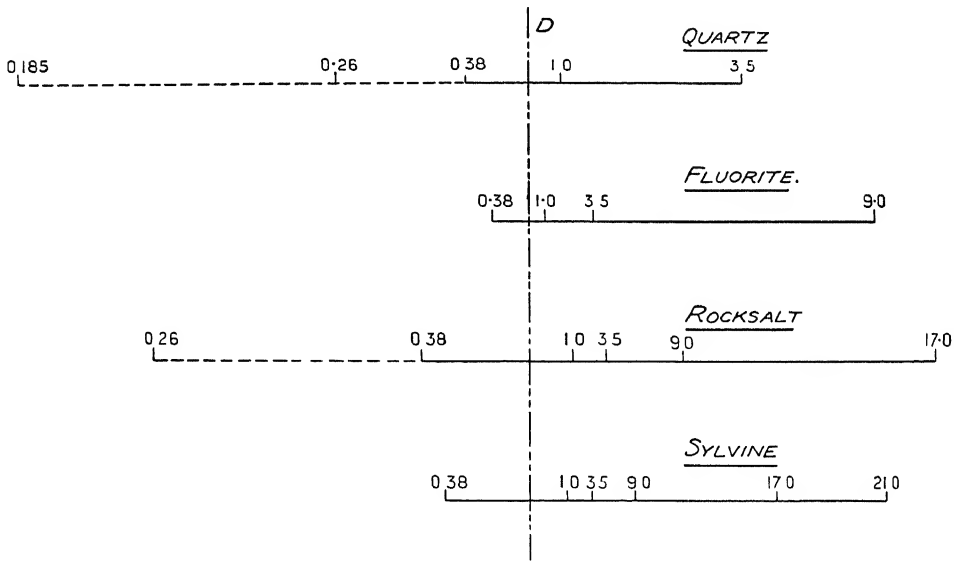


FIG. 41

[1] The calibration can be extended to 0·26 μ in the ultra-violet.

Here is, perhaps, a suitable opportunity for giving a warning concerning the effect of scattered radiation in infra-red work. There is one source of radiation which is more useful in this region than any other, the Nernst filament (F 354). The Nernst filament disappeared from commerce on the introduction of metal filament lamps, and it is only after years of waiting that a source of supply once more became available for scientific purposes. The Nernst filament gives a radiation whose intensity varies rapidly with wave-length. The intensity is very great at 2 μ, but one may be working in regions where the radiation is feeble ; measurements of great interest to chemists have recently been made in the neighbourhood of 10 μ, for instance, and in such a case the amount of energy of the wave-length indicated on the drum is a very minute fraction of the total energy entering the instrument. If this feeble energy is still further reduced by a considerable amount of local absorption at this wave-length, diffused radiation from the whole remaining spectrum may get through the second slit in sufficient quantity to prejudice the accuracy of the measurements. The remedy in such a case is to interpose, outside the instrument, between the light source and the first slit, either (if it can be found) a filter which cuts off most of the spectrum which is not at the moment under observation, or failing that, a simple prism and lens arrangement which will throw on the slit only the part of the spectrum under observation. Best of all is a double monochromator such as that described on p. 81.

Valuable and detailed accounts of methods employed in the measurement of absorption curves of gases have appeared in the following recent papers :

(1) Robertson and Fox, *Roy. Soc. Proc.*, *A*. **120**, pp. 128-157, 1928. (The infra-red absorption spectra of Ammonia, Phosphine and Arsine.)

(2) Bailey, Cassie and Angus, *Roy. Soc. Proc.*, *A*. **130**, pp. 133-141, 1930, (and a number of later papers also in *Roy. Soc. Proc.*).

The former paper deals also with the general arrangements of apparatus, including source of radiation and absorption tubes, conditions affecting the thermopile, and choice and mounting of the galvanometer. The method of calibrating the spectrometer is also described together with the effect of change of temperature and the routine of taking readings is described

The latter series of papers supplements the information given in the former. In particular, arrangements for supporting and shielding the galvanometer are described which, together with suitable shielding of the instrument, permit of its being worked even at the top of a building subject to vibration, magnetic disturbance and draughts. The errors attendant

upon uncertainty in drum reading, minimum deviation setting, prism angle and temperature of the prism are discussed, together with the accuracy to be expected from the four different prisms.

The following remarks are summarised from the recommendation in these papers.

Light Source.

A Nernst Filament (F 354) run at a constant voltage is most suitable. In order to maintain the necessary constancy of output it can be run from a suitable secondary battery with a regulating resistance and voltmeter for control of voltage fluctuations which are present. The accuracy of readings is very largely dependent on the constancy of emission of the source.

Absorption Tubes.

The absorption tubes must be gas tight and should have end plates of quartz, fluorspar, or rock salt according to the spectral region under investigation. Their lengths will depend on the nature of the substance investigated. In the work (1) cited above tubes of 100 mm. and 450 mm. length were used. (The gases investigated were ammonia, phosphine and arsine.)

Galvanometer.

A Paschen type of galvanometer (F 441) was employed and in conjunction with adequate screening and insulation from vibration was found satisfactory. (The latest practice favours some kind of thermo-relay, which involves the use of two galvanometers preferably of the moving coil type.)

Enclosure of Apparatus and Precautions against Disturbances.

It is necessary to place the apparatus in separate heat insulating enclosures. The spectrometer is best housed in a chamber which can be maintained at a constant temperature and can be freed from moisture, such as might attack the prism, by means of a suitable drying agent.

The light source should also be contained in an enclosure in order to prevent stray radiation affecting other apparatus and to secure constancy of radiation by shielding it from draughts.

It has been found that rapid variations of pressure (even of small magnitude) cause adiabatic heating of the thermopile.[1] It is therefore enclosed in an airtight housing which can be evacuated if desired. As a further precaution (against currents induced by swinging in the earth's

[1] This was pointed out to the present writer by Professor Paschen in 1911.

field) the conductors between the thermopile and galvanometer may be passed through glass tubes secured to the benches.

In order to avoid heating the spectrometer prism by the proximity of the observer, the wave-length drum may be rotated by suitable low gearing and its position read by means of a telescope.

Methods of taking Readings.

Two principal methods are in use. In one a complete set of galvanometer readings at various parts of the spectrum is taken with the absorption tube filled with the substance under observation. Subsequently the tube is carefully emptied and an exactly similar series of readings is taken with the empty tube (Coblentz [1] and others).

In the second method readings are taken alternately through the filled tube and either (*a*) an exactly similar empty tube (Robertson and Fox [2]) (*b*) a pair of end plates similar to those used in the first tube (Imes [3]).

It is claimed that the second method has the advantage that readings are taken with the gas tube and control tube with only a short interval between, thus reducing the likelihood of errors arising from fluctuations in the light source, and the chances of error in repeating wave-length settings are avoided.

Two workers may conveniently carry out the measurement. One takes the readings of the galvanometer deflections while the other alternates the observation tubes, operates the light source shutter, maintains the voltage on the light source at a constant value, and sets the wave-length drum.

Effects of Infra-Red Radiation on the Solutions.

As in the case of ultra-violet spectrophotometry it may occur that a substance shows a tendency to alter in nature when subjected to the full radiation of the light source. This is a temperature effect, and when this occurs it is often possible to avoid it by putting the absorbing substance after the infra-red spectrometer instead of in front of it.

More Powerful Instruments.

The infra-red spectrometer described above is made in two sizes, D83 and D88, the latter having a larger aperture than the former. Though the larger aperture instrument has somewhat less dispersion than the other, the larger aperture makes it possible, if desired, to work with a smaller slit, so that finer detail can be observed. It was the D88 with

[1] Investigations of Infra-red Spectra : Carnegie Institute of Washington, 1905.

[2] *Proc. Roy. Soc*, *A* 120, 128-148 (1928).

[3] *Astrophys J*, Vol. 50, p 251 (1919).

rock salt prism which was used by Robertson and Fox in the paper cited above.

If a larger dispersion is required the double monochromator already described, D 107, when fitted with a thermopile is to be recommended. Its dispersion is approximately equivalent to that of four 60° prisms, and its focal length is one and one-third times that of the D 88.

The rotation vibration bands of ammonia, phosphine and arsine resolved by Robertson and Fox (*loc. cit*) extend from 3μ (using quartz), from 6μ (using fluorite) and from 8μ (using rock salt)—towards the longer wave-lengths in every instance. To attain this result it was necessary to drive the care in experimenting to the extreme of what is possible, and it is safe to say that for determining rotation-vibration bands in the infra-red the double monochromator D 107 should be recommended.

A still more powerful instrument is available, the infra-red prism-grating spectrometer D 79, which has been developed from the type described by Śleator (*Astrophys. J.*, **48**, 125-143, 1918) and Imes (*Astrophys. J.*, **50**, 251-276, 1919) with modifications and improvements based on the suggestions of Snow and Taylor (*Proc Roy. Soc.*, *A*. **124**, 442-452, 1929). Essentially it consists of a grating spectrometer used in train with a monochromator having a large aperture rock salt prism. The combined instrument is built into a substantial cast metal casing (see Fig. 39) provided with cover plates for total enclosure of the optical system. Polished stainless steel mirrors are used throughout (1, 6, 8).

A Nernst glower 9 is attached to the instrument. Its radiations pass through either of two absorption tubes, 10, are reflected into the prismatic monochromator and thence pass to the grating spectrometer.

The absorption tubes for gases are each of 2·5 cm. internal diameter and 45 cm. long. They are of brass and have rock-salt end plates, held in place by screw-on end caps. A simple rocking device enables them to be interchanged quickly and in correct alignment.

The slits 11, 12, 13 are of the symmetrical type with an accurate differential screw motion (with 100 and 50 threads per inch), controlling the opening of the jaws, which are of stainless steel.

The rock salt prism 7, has an aperture of 11½ cm. by 9½ cm. and a refracting angle of 18°. It is fitted in a mount provided with geometrical locating devices, so that it may be removed and replaced without affecting the adjustment. A plane stainless steel mirror 6, is mounted on the prism table, behind the prism, and is provided with a new type of fine adjustment which permits of very precise control

A helical drum 5, calibrated directly in wave-lengths from 0·38μ to 17μ rotates the prism table by means of an accurate micrometer screw, similar to that used in large wave-length spectrometers (D 10)

The gratings, of which any one of three with alternative rulings may be employed, are mounted in a substantial three-sided turret, 3, carried on a finely divided circle. Each grating is fitted with a quickly removable cover protecting it from damage when not in actual use. Fuller details will be found in the Publisher's catalogue D.

Thermopiles.

The thermopile customarily used with the Hilger infra-red spectrometers is the Hilger Sensitive Thermopile (F 100, etc.) and this was the type used by Robertson and Fox (*loc. cit.*) These are more sensitive than any with which we have compared them, with the sole exception of the Müller thermoelements (F 766, etc.) which are five to seven times as sensitive. Now that the study of fine structure in the infra-red has assumed such importance it seems likely that the Müller thermoelements, used with a Müller-Hilger double monochromator will meet a distinct need.

MEASUREMENT OF THE RAMAN EFFECT

As was explained in Chapter III, it is often possible to obtain information from a study of the Raman Effect which it is not possible to derive from direct observations of infra-red spectra. There is therefore a growing practice to investigate the two phenomena simultaneously, and it will not be out of place to include in this chapter a brief account of instruments of suitable design which are available for photographing Raman spectra.

A few practical points in connection with the Raman effect may first be considered The effect is essentially one of scattering by molecules in perfectly transparent substances (*e.g.* distilled water) and consequently is very faint. It therefore requires for its investigation as powerful a light source as possible combined with efficient means of concentrating the light within the specimen

One method of illumination frequently adopted is that due to R. W. Wood A long column of the material is placed in line with the axis of the spectrograph collimator, and parallel to it are placed one or two lamps of similar length

The intensity of the scattered light is proportional to the effective length of column and thus if the incident light be concentrated at the centre of the specimen, little is to be gained by using an instrument with a collimator of high aperture ratio. The reverse is, however, the case with the camera

For a given size of optical component, the attainment of light-gathering power must necessarily be accompanied by decrease of dispersion, while to increase the size in order to retain dispersion is expensive.

The usual way of compromising is to use two or three instruments of varying apertures and dispersions. One of large aperture and small dispersion is used in preliminary work to determine approximate positions of bands and necessary exposures. A slower instrument adjusted for the region of interest may then be substituted and an exposure given which can be well forecast from the preliminary results. If desired this procedure may be taken one stage further with an instrument of still higher dispersion.

A quartz spectrograph (E 420) with camera of f/3 aperture is available, and also the same instrument with optical parts of glass instead of quartz (E 439).

When the above instruments are considered to be of insufficient dispersion, recourse must usually be had to those of smaller relative aperture and the necessity for correspondingly increased exposure faced. The E 2 spectrograph has been successfully employed for this work, while the E 315 instrument is to be preferred. The large E 1 spectrograph has also been used, but this will only be desirable in special cases.

The decision as to whether a quartz or a glass spectrograph shall be used will rest upon the choice of exciting radiation. The intensity of scattered light, in accordance with Rayleigh's law, is proportional to the inverse fourth power of the wave-length, so that an intense isolated ultra-violet line is often to be preferred. This is not the case, however, if (*a*) the substance shows absorption in the neighbourhood of this line, (*b*) the substance is fluorescent and the fluorescence masks the Raman lines, or (*c*) the substance suffers photo-chemical change.

A brief account of such work, including a short bibliography, is given in "The Raman Effect in Organic Substances and its Use in Chemical Problems"—A. Dadieu and K. W. F. Kohlrausch, *Journal of the Optical Society of America*, 21, 286-322 (May 1931), while the publication by J. Springer, Berlin, of a book by Kohlrausch on the subject (*Der Smekal-Raman Effekt*) provides a valuable source of reference on the present theory of the subject.

APPENDIX 1

TABLE I.

γ VALUES FOR VARIOUS PLATES, OBTAINED BY USING THE DEVELOPERS, 1, 2, 3 DESCRIBED BELOW AT 65° F.

Time of development (minutes at at 65° F.)	γ values obtained for light of the quality specified			
	White light; colour temperature 2360° K. (vacuum lamp)	White light (2360° K.) screened by filters		
		Tricolour blue 3900–5150 A.	Tricolour green 4800–6100 A.	Tricolour red 5800 A.–red end
	Ilford Panchromatic Half-tone Developer No. 2 . caustic potash, hydroquinone			
2	6–7	5–6	6–7	8·5–9·5
3	7–8	6–7	8–9	9–10
	Ilford Rapid Process Panchromatic Developer No. 2 : caustic potash, hydroquinone			
2	3–4	2·5–3·5	2–3	3–4
3	4–5	2·5–4	3–4	4–5
	Wellington Soft Spectrum Developer No. 1 . metol, hydroquinone			
5	1 ·1·5	0·6–0·9	1–1·5	1–1·5
7½	1 25–1·75	0 80–1 2	1·25–1·75	1·25–1·75
	Ilford Hypersensitive Panchromatic Developer No. 1 metol, hydroquinone			
5	0·5 0·7	0·4 0·6	0 5–0·7	0·5–0·7
7½	0 6 0 8	0·5–0·7	0·6–0·8	0·6–0·8
	Ilford Special Lantern plate Developer No 3 Exposure to light from unscreened vacuum lamp at 2360° K.			
2	1·75–2 0			
3	2·00–2·5			

We have to thank Ilford, Ltd , for very kindly supplying the information contained in the above table

TABLE II.

METHODS OF DEVELOPMENT.

(1) Metol, hydroquinone.

Metol	2 gm.
Sodium sulphite (crystals)	100 gm
Hydroquinone	8 gm.
Sodium carbonate (crystals)	100 gm.
Potassium bromide (10 per cent. solution)	8 c.c.
Water up to	1000 c.c.

For use take 1 part with 2 parts of water.

(2) Caustic potash, hydroquinone.

A. Caustic potash	50 gm.
Water up to	1000 c.c.
B. Hydroquinone	25 gm.
Potassium metabisulphite	25 gm.
Potassium bromide	25 gm.
Water up to	1000 c.c.

For use take equal amounts of A and B.

(3) Developer for black tone (rapid) lantern plates.

A. Hydroquinone	9 gm.
Sodium sulphite (crystals)	50 gm.
Water up to	500 c c.
B. Caustic soda	5 gm.
Potassium bromide	2 gm.
Water up to	500 c c.

For use take equal amounts of A and B.

EASTMAN SCIENTIFIC PLATES

A unique range of spectroscopic plates has recently been made available by the Eastman Kodak Company and has been described by Mees (*J.O.S.A.* and Publications of the Eastman Kodak Co.) It includes a number of infra-red sensitizings with one of which wave-lengths as long as 12,000 A can be recorded Although the other sensitizers do not record such an extended range, they are more sensitive within their own range. A particularly useful plate (L) is one which has uniform sensitivity throughout the visible and is sensitive to 9000 A without any insensitive region. Each sensitizer is available in a number of different types, that is, emulsions of different speeds It may be stated in general that the higher the speed the less the contrast and the larger the grain.

Reduction and Intensity.

If still greater contrast is required, it may be obtained after thorough fixation by reduction with Farmer's reducer The plate is immersed (after washing) in a solution of sodium thiosulphate to which has been added sufficient potassium ferricyanide to colour the mixture a deep yellow. By this means the density of all parts of the plate is reduced by the same amount, the effect being to increase the density ratio. The

reduction also has the effect of removing the stains produced on the long development. The resulting plate has now a greatly enhanced contrast, but, by subsequent intensification, still greater contrast may be obtained. One worker[1] has tried many formulae without success, but finds the following gives uniformly satisfactory results.

The plate after washing is steeped in a solution of the following composition :

Mercuric Chloride - - -	25 gms.
Potassium Bromide - - -	25 gms.
Water to 1000 ccs.	

until all parts have been bleached. It is then removed and washed for five minutes, and intensified in a solution made up in the following manner :

Silver nitrate - - - -	25 gms.
Water - - - - -	250 ccs.
Potassium cyanide - - -	25 gms.
Water - - - - -	250 ccs.

The silver solution is slowly added to the cyanide solution with stirring, and the mixture filtered.

The image reappears, and the plate may remain for about fifteen minutes in this bath for completion.

This formula does not appear in the standard works, and requires careful preparation and use. The plates so treated deteriorate and are useless after three or four weeks, but the accuracy of reading is greatly increased

A variation of " Farmer's Reducer " which has been found in the Hilger laboratories to give good results is as follows :

SOLUTION A.

Potassium Ferricyanide - -	5 grams ($1\frac{3}{4}$ ozs.)
Water - - - - -	100 ccs. (35 ozs.)

SOLUTION B.

Hypo - - - - - -	20 grams (7 ozs.)
Water - - - - - -	100 ccs. (35 ozs.)

After developing, fixing and washing the plate, it should be immersed in Solution A for from thirty to ninety seconds. Rinse immediately in water and then immerse in B for ten minutes A thorough wash then completes the process.

[1] Dr R Macwalter, Biochemical Laboratory, University College, London.

APPENDIX II

THE DETECTION AND INVESTIGATION OF POISONS AND THE CONTROL OF PURITY IN FOODSTUFFS BY SPECTROSCOPY

FOR practical toxicological work absorption and emission spectrography are of importance, and a section dealing with applications of emission spectrography has therefore been added to the present Appendix, although the actual methods to be adopted for emission spectrography are fully dealt with in a companion book, *The Practice of Spectrum Analysis.*[1]

Organic poisons may frequently be identified by their absorption spectra, as determined by a quartz spectrograph in conjunction with a photometer. The researches of Dobbie and others [2] have shown that each alkaloid exhibits a characteristic curve, as also do the glucosides and other principles.

The chief preliminary is the extraction of the active principle in a state sufficiently free from impurity. The usual procedure of separation for an alkaloidal assay is followed up to the point where the alkaloid is obtained in acid aqueous solution by shaking out from the immiscible solvent; then, instead of proceeding to weigh and identify the principle by chemical tests, the solution is introduced into an observation cell, examined spectrophotometrically, and the curve of extinction coefficients plotted. The form of the curve identifies the substance and the height of the ordinates expresses its concentration; whence, if quantitative relations have been preserved in arriving at the solution, the proportion in the original substance may be calculated. The quantity of active principle required is small, as illustrated by quinine hydrochloride, which gives a curve rich in detail when observed with a 2 cm. cell holding 3 or 4 c.c. of a solution containing only 0·0083 per cent. of the salt, that is, with 0·3 mgm.

In practice it is desirable to eliminate any of the immiscible solvent remaining in the aqueous layer if, as with benzene, traces of it are likely to affect the absorption curve. This may be achieved by shaking the acid aqueous separate with a little ether, which gives no absorption such as can confuse the search.

[1] Published by Adam Hilger, Ltd. [2] *J.C.S.*, p. 508, 1903.

With regard to an atlas of absorption spectra of the alkaloids, the reader will find some information in *International Critical Tables*, V, pp. 361-379. The work of Dobbie, Fox, Lauder and Tinkler[1] is helpful, but the work was done before the advent of spectrophotometers for exploring the ultra-violet region, with the result that the curves are obsolete, lacking as they do quantitative value and being misleading when work done with a spectrophotometer is under consideration. However, useful and suggestive information is to be derived from the latter half of *die Physikalische Chemie der gerichtlichen Medizin und der Toxikologie mit spezieller Berücksichtigung der Spektrographie und der Fluoreszenzmethoden* by Dr. Hans Fischer (Zürich, 1925). This book gives absorption curves for several toxicological substances. In practical search for the alkaloid or other active principle in a plant or other material, one has to deal with the aqueous alkaloidal solution obtained in the usual separation, preferably produced according to some process regularly adopted in the laboratory. By making a series of spectroscopic examinations of such solutions of constituents, an atlas can be compiled which will be far more useful than any published atlas can be. The production of half a dozen or a dozen curves in this way using material of known character will prove the best means of acquiring the necessary experience for dealing with unknown materials.

Again, it has to be borne in mind that the alkaloid as separated in analytical practice is rarely pure; it is more often a mixture characteristic of the drug rather than of an academically pure chemical.

Two or three examples recently published will be suggestive of some wider application in the field of inquiry cultivated by the individual worker.

The Alkaloids of Ergot have always presented difficulty to the toxicologist. The following is reprinted from the *Pharmaceutical Journal* of March 16th, 1929, page 260.

> "Ergotinine, ergotoxine, ergotamine, and ergotaminine, the four known alkaloids of ergot, show almost identical absorption in the ultra-violet, with a maximum of 316$\mu\mu$ and minimum of 272$\mu\mu$. The alkaloidal content of ergot may be determined by comparing the absorption of the alkaloidal fraction of the extract with that of a solution of ergotamine of known concentration. The Keller-Fromme method was used for the extraction of the alkaloids, except that one per cent tartaric acid was substituted for 0·25 per cent. hydrochloric acid. The alkaloidal solution thus obtained, after filtration and dilution when necessary, was photographed through the spectroscope in layers 2-40 mm., and the concentration determined by comparison with a similar spectrogram of a solution containing

[1] *Loc. cit.*

4 mgm. of ergotamine in 50 c.c. of one per cent. tartaric acid. The result obtained in this way compared fairly well with parallel determinations made by the chemical method of Keller and the physiological method of Broom and Clarke.—A. Harmsma (*Pharm. Weekbl.*, 65, 1928, 1114; through *Chem. Abstr.*, January 10, 1929, 235)."

It is well known that the form of the absorption curve of an alkaloid in a variety of circumstances is usually constant, although the extinction coefficients may have other but equally definite values according to whether the base is hydrated or not. Thus, for nicotine, Lowry and Lloyd (*J.C.S.*, 1376, 1929) record the following figures:

Nicotine in water	-	-	Log $\epsilon = 2{\cdot}49$ at 2640 A.
,, *cyclo*hexane	-	-	,, 3·43 2650 A.

It is suggested that the aqueous solutions may contain an un-ionised hydrate or pseudo-base.

The Control of Purity in Foodstuffs.

Absorption Spectra in the Visible Region (colouring matter in foodstuffs).

The identification of natural and artificial colourings used in foodstuff and similar industries may usually be conducted by means of a visual spectroscope and a Baly tube (F 18), but the work may be conducted more precisely by means of the visual spectrophotometer. Help will, of course, be derived from the absorption spectra of various colours described in the literature, but the most practical and simple method is to work by comparison with standard solutions, since the spectra of most of the natural colours derived from fruits and other foodstuffs, and those attributed in the literature to dyes of a given name, are rarely described with sufficient detail and under such practical conditions as to make them reliable for the practical chemist.

The "*Colour Index*" published by the Society of Dyers and Colourists gives the wave-lengths of the principal bands of the majority of the dyes.

Absorption Spectra in the Ultra-Violet Region.

These may be studied by means of a quartz spectrograph and a spectrophotometer and the method may be applied to coloured and colourless bodies alike. Benzoic acid, salicylic acid, plant and animal principles, (whether protein, glucosidal, basic, ketonic or otherwise in character), colourings, essential oils, and animal extracts, all give well-characterised absorption spectra and may usually be identified when only very small quantities are present. Using a 1 cm. cell one usually works with

solutions having a concentration of one in a thousand to one in a hundred-thousand.

A feature of such work which is not usually appreciated especially by those systematically trained in research, is that empirical work is often of far greater practical utility than work done on more systematic lines. For example, it will be found that the one per cent. solution of a " straight " jam from a given fruit will afford absorption bands of a particular character, while similar solutions from other fruits will give bands exhibiting other features. So long as it is found that these peculiarities are constant, it often matters little, in practical work, whether a scientific basis for them can be found in the first and practical instance.

APPENDIX III

EMISSION SPECTROGRAPHY AS APPLIED TO PROBLEMS OF INTEREST TO THOSE PRACTISING ABSORPTION SPECTROGRAPHY

In this section some applications of emission spectrography to problems whose nature brings them within the purview of those who principally make use of absorption spectrophotometry will be briefly discussed. Little attempt will be made to describe general methods or apparatus as these are fully dealt with in the publications of Adam Hilger, Ltd., entitled *The Practice of Spectrum Analysis* and *Spectrographic Outfits for Metallurgical Analysis*. The authorised translation of vol. II. of *Die Emissions-spectralanalyse* by W. and We. Gerlach (Adam Hilger, Ltd.—in the Press) gives many examples of the detection of metals in animal tissues.

THE DETECTION AND INVESTIGATION OF POISONS BY EMISSION SPECTROSCOPY

Applications.

The knowledge that ordinary spectroscopic technique is capable of determining with certainty the presence of any of the great majority of the elements, when these elements occur even in minute quantity in complex material, must at an early stage in the history of spectroscopy have prompted many an enterprising worker to consider the possible application of this branch of practical science to the purposes of toxicology. In the early days, the scope of successful experiment was so limited that it is not surprising that the method found little favour · but during the

last thirty years the limitations have been gradually swept away, until to-day it is true that no metallic element can escape detection by the modern quartz spectrograph or, with certain exceptions, by the visual spectroscope reading directly in wave-lengths. Of the non-metals, arsenic is the only one which is usually of direct forensic interest, and normally this is detected under the same spectroscopic conditions as are the metals, save only that for this element one must work in the ultra-violet.

The subject lends itself to the following classified treatment:

(1) *Metallic poisons which are not readily volatilised when the suspected material is ashed.*

Besides metallic poisons, the detection of an innocuous metal is often of determinative value in toxicological investigation. Identification of the metal itself may be the object sought, but not infrequently the innocuous metallic elements found may give the clue to the anions or organic principles responsible for the cause of the inquiry.

In the preliminary experiments it is highly important to ascertain the whole range of metallic elements present in the animal tissues or in the foodstuffs or other material under suspicion, and it is desirable that the information shall be (*a*) so comprehensive as to include the unsuspected as well as the suspected; (*b*) so conclusive as to leave no doubt as to whether the result is positive or negative; (*c*) rapidly obtainable. Moreover, it is of very great advantage to get these preliminary fundamental problems solved with a minimum quantity of material, so as to leave practically the whole available for quantitative work and further investigation.

All these advantages are realisable when spectroscopic methods are employed. A gram or even less of an animal tissue, body fluid, suspected food, medicine, etc., will usually yield on careful ashing sufficient of the significant element to be identified by the spectrum, and to show whether the proportion present is much, a little, or merely a trace.

The recognition of phosphorus is of little consequence in itself, but when its presence is taken into consideration with that of certain other elements revealed by the spectrum, it may be directly suggestive. For example, in the case of a medicinal syrup, feeble *phosphorus* lines from a trace of ash which indicates no other element would lead to the inference that the preparation might be the official syrup of Codeine Phosphate. With strong phosphorus lines and a large number of fairly strong iron lines accompanied by no others, there is suggestion of strychnine in the form of Easton's Syrup, while with much phosphorus and several other elements one of the many compound phosphate or hypophosphite syrups would be indicated. By studying the spectrum so as to discover

all the metallic elements indicated, and by giving some attention to the relative intensities of the lines, the formula of the syrup may be approximately derived, in so far as its mineral constituents are concerned, and this at once prompts a search for likely alkaloids or other active organic principles.

Elements closely allied chemically cannot be confused spectroscopically. The lines for arsenic and antimony are far apart in the spectrum, as are those of lead and bismuth, tin and cadmium, barium and strontium, and others which are often the cause of difficulty when mere traces have to be separated and identified by chemical means.

Boric Acid affords a striking illustration of two of the principles here enunciated, namely the significance of an apparently innocuous element, and the discovery of an unsuspected element. Both in England and America several cases have quite recently come to light in which death has been caused by overdosage with boric acid. It is now suggested that many cases of obscure illness may be attributed to boron poisoning (*B.M.J.*, 1928, I. 176 ; *J. Amer. Med. Assoc.*, 1928, 90, 345).

Aluminium may not be regarded as a toxic agent, but it receives spectrographic notice in *Science*, vol. lxix. Feb. 15th, 1929, at the hands of Kahlenberg and Closs in the following terms: "We have checked the work of McCollum and co-workers in the laboratory and have found that they are quite in error. Using the Hilger quartz prism spectrograph, as they did, we found aluminium to be present in egg, (*and numerous other biological materials*).... The complete experimental details will soon be ready for publication." McCollum (*J. of Biolog. Chem.*, 1928, 77, 753), using a Hilger E 1 spectrograph, found aluminium absent from most, but not all, natural tissues, but detected 1 part in 2,000,000 satisfactorily.

In discussing quantitative spectroscopy in the *J. Soc. of Dyers and Colourists*,[1] Dr. S. Judd Lewis says:—"A very recent series is that of the proportion of aluminium in biological material. The figures for three specimens will indicate the degree of accuracy attained, they refer to parts of aluminium in one-million parts of the material.

	1	*2*	*3*	*Mean*
Specimen *A* - - -	0	0	0	0
,, *B* - - -	0 3	0 4	0 2	0 3
,, *C* - - -	1 2	1 1	1 3	1 2"

(2) *Metallic poisons which are volatilised by ashing.* These require special care in conducting this process, according to their nature. *Arsenic* especially calls for consideration here, but it may usually be held back

[1] *J. Soc. Dyers and Colourists*, vol. xlii., Dec. 1927.

by ashing with a nitrate (adding a little nitric acid to form nitrates with the bases in the ash) so as to oxidise it to arsenate. However, arsenic being a non-metal, minute traces of it are not so certainly detected spectroscopically by the emission method; and if only traces are suspected, it is better to search by the Gutzeit or Marsh-Bezelius chemical method.

Mercury, which is exceptionally volatile, can be detected spectroscopically when present in only minute amount. Quantities such as 0·000001 gram are determined spectroscopically in chemical, explosive and other practice, while its application to pharmacology is well attested.

Mercury, if not too small in quantity is detectable in the ordinary course of arc or spark analysis, but when it occurs diffused through a mass of organic substance, a special technique is necessary. This depends upon the conversion of the compound into perchloride or some similarly volatile compound, the volatilisation thereof in a suitable modification of the gas vacuum tube under reduced pressure and, at the temperature of hot water, visual observation of the spectrum when the vapour in the tube is excited by the electric discharge. The procedure is quite convenient for regular work with any number of specimens when once the equipment is installed. The test is extremely delicate, 0·000001 or even 0·0000001 gram being detectable, and hence it is applicable to studying the migration of the element through the organism. In modified form the test was extensively applied to toxicology by K. C. Browning in Ceylon. He used a Hilger spectroscope with scale. To take one example, 10 c.c. of urine was electrolysed in a small quartz dish for 10 or 15 minutes, using 0·2 ampere, and then the miniature gold foil electrode on which the mercury was deposited was heated gently in a vacuum tube, and the spectrum produced was observed in the ordinary way. "It is thus possible to detect one part of mercuric chloride in one hundred million parts of urine, using only 10 c.c. of urine"; thus one ten-thousandth part of a milligram is discoverable. He says, "The method now described is easy to work," and that "if 0·06 gram of mercuric iodide is injected intravenously, mercury can be detected up to three days in the urine" Stomach washings and other material may be similarly examined. (*J.C.S.*, p. 235, 1917.)

EMISSION SPECTROSCOPY IN CONTROL OF PURITY IN FOODSTUFFS

The subject may be considered in two divisions :—

(*a*) Emission Spectroscopy as applied to the examination of Inorganic Materials.

(*b*) Emission Spectroscopy as applied to the examination of Organic Materials.

These applications of Emission Spectroscopy to the Foodstuffs industry are actually employed by the consulting chemists to Adam Hilger, Ltd. in their practice, and it is they who have prepared the following notes.

(a) Emission Spectroscopy as applied to the examination of Inorganic Materials.

This method can be applied very widely indeed to every variety of inorganic material. Usually very little preparation of the material is necessary before placing a small quantity of it upon the top of the lower copper electrode of a simple arc lamp which has been placed in position in front of the slit of the spectroscope or spectrograph. The current is then switched on, the arc struck, and the spectrum of the light emitted is examined either visually or photographically according to the type of instrument employed The spectrum is composite, that of the material under examination and that of the copper of the electrodes. The copper lines serve for calibration purposes. In this simple way one may submit to critical analysis any metal or other inorganic material, for example filtering powders, such as kaolin, kieselguhr, etc., for an unusual or disturbing element ; or earthenware or porcelain apparatus, enamelled ware, glass, and all kinds of salts, either for their complete analysis or for any specified element such as lead, tin, arsenic, or other metallic impurities, whether poisonous or innocuous.

In the case of metals, such as those occurring in tins used for packing, storage tanks, manufacturing apparatus, machinery, etc., the sample may be presented in the form of scrapings from the surface, filings, chippings or the like. Since the quantity of metal required is only a few milligrams, even a single milligram, the necessary material can usually be removed from the apparatus or machinery without causing sensible damage.

When the metal is available in the form of a piece, the examination may be made directly, using the fragment as one electrode, and a copper rod as the other electrode , or if desired a second piece may replace the copper, so that the spectrum is free from intruding copper lines. This alternative would be useful when looking for copper itself ; or a silver electrode (U.S. brand) may replace the copper one.

It should be appreciated that the value of search for traces of foreign elements is not confined to the discovery of objectionable impurities in the foodstuffs themselves; other economic values appear. For example, metals may be subject to local corrosion due to segregation of one of their constituents and other like effects, as is the case with lead in zinc, or arsenic in tin. Thus it becomes possible to remove from the surface a few milligrams of the metal at the point of corrosion, and a few milligrams at a point where the metal is sound, and to compare their spectra, and so to ascertain whether the disturbance is due to foreign elements or not. By the arc method arsenic is easily detected when present to the extent of 0·01 per cent.

(b) Emission Spectroscopy as applied to the examination of Organic Materials.[1]

This is usually best conducted on the ash of the material or, in the case of a liquid, on the total solids obtained by evaporation and then ashing in the usual way, adding a suitable reagent to fix volatile elements when necessary, for example, nitric acid to oxidise and so retain arsenic or phosphorus.

A little of the ash is then examined spectroscopically, exactly as already described for the particles of metal. All the metallic elements, as also the non-metallic elements, silicon, boron, arsenic, phosphorus, will reveal themselves when present, even if only in slight traces.

Happily those elements which are of first practical interest in the food industry when present in only small quantities, such as copper, lead, zinc, barium, tin, antimony, are very easily recognised by their characteristic strong lines and are much more readily detected than are traces of some of the elements popularly associated with spectroscopy, such as potassium. Search for copper, zinc, etc., in peas and similar foodstuffs may be made under similar conditions.

In a recent investigation it was shown that lead is detectable in 10 milligrams of ash derived from animal material, when present in the proportion of only one part in 10,000 parts of the ash, and that with care this sensitiveness may be multiplied; also, that in the case of zinc a quantity estimated to be considerably less than the one hundredth part of a milligram was readily detectable in a milligram of ash obtained from organic material of quite another character.

Hence, traces of any of the metals in foodstuffs derived from containers, whether of metal, enamel, earthenware (glazed or unglazed), or glass, etc., which may yield any of their normal or occasional elements to the material stored in them, can be detected when present in only slight traces and

[1] The spectrum analysis of animal tissues in clinical and pathological work is very fully dealt with by W. and We. Gerlach (*loc. cit.* p. 119).

approximately estimated by the ordinary spectroscopic or spectrographic procedure.

An advantage of negative character, although no less valuable on that account, is the confidence inspired by a negative result. If the element cannot be found spectrographically it is certainly not present in any significant proportion, even when that proportion is much smaller than that detectable by the usual chemical methods.

It is usual to use copper electrodes, but of course copper is then not detectable in the material. This difficulty is avoided by using the special copper-free silver electrodes of the quality sold under the series of Hilger Spectroscopically Standardised Substances, H.S. Brand.

OTHER APPLICATIONS OF EMISSION SPECTROSCOPY

It will readily be appreciated that the utility of Emission Spectroscopy does not lie solely in its applications to toxicology and the purity control of foodstuffs. In most laboratories the need will more or less frequently arise for delicate means of detecting metals which when present even in very small quantities have important effects upon the subjects under study. For such purposes spectrography is unrivalled in its usefulness, and since a great part of the equipment used for absorption spectrophotometry is suitable also for emission spectroscopy it is appropriate that it should be employed collaterally with absorption methods.

Applications for it are found in fields akin to biology and pathology. Hugh Ramage and J. H. Sheldon,[1] using an interesting flame method, found appreciable amounts of barium in the choroids of the eyes of oxen. Zbinden,[2] using an arc between carbon electrodes, into which the substance was introduced, found that aluminium, chromium, copper, iron, manganese, lead, tin, titanium, vanadium and zinc are normal constituents of human and cow's milk in constant proportions. Investigations on milks have also been made by Norman C. Wright,[3] by a spark method, and Ramage.[4]

According to Ramage's flame method the material is first minced, dried and powdered in an agate mortar. A weighed quantity is then rolled into an ashless filter paper which is burnt completely in an oxy-coal gas flame in front of the slit of the spectrograph using a quartz lens to focus an image of the flame on the slit. Fluids are pipetted in known quantities on to the filter paper rolls.[5] Methods are described for obtaining quantitative

[1] *Nature*, Aug 29, 1931 [2] *Le Lait*, 11, No 102, pp. 113-124, 1931.

[3] *Science*, 69, page 77, 1929. [4] *Nature*, 20th April, 1929.

[5] H M Fox and H Ramage, *Nature*, 126, p. 682, Nov. 1, 1930, and *Roy. Soc. Proc., B* 108, 157-173, 1931.

results. In the paper cited investigations are made of a number of annelids in which the whole animals are analysed, and molluscs, the separate organs of which are dealt with.

The same method has been used in the examination of mushrooms[1] in which more than 0.01% silver as well as copper, lithium, iron, potassium and calcium were found. In an earlier paper,[2] investigations on materials such as wheat and other cereals are summarised.

THE SPECTROGRAPHIC EXAMINATION OF BODY FLUIDS FOR INORGANIC SUBSTANCES

For the spectrographic determination of lead in urine, several methods besides that of the arc between graphite electrodes could be used. The following methods are suggested, and these have the advantage that no preliminary chemical treatment or separation is necessary before the spectrographic test.

1. Spark with the liquid under test.

For this method either the Pollok sparking tube or the modern improved sparking tube designed by Dr. C. Stansfield Hitchen (F 638) can be used. The latter is designed to prevent decomposition of the liquid or incrustation of the electrodes. A spark is obtained between the liquid (fed continuously through a capillary jet) and an upper electrode of gold or graphite of high purity

According to W. N. Hartley (see " Wave-length Tables," 2nd Edition, p. 69) who used graphite electrodes moistened by the liquid under test, 0.01% lead can be detected.

2. Flame Spectra.

A new technique, using flame spectra, has been devised by Prof. H. Lundegardh,[3] who describes the application of the method to biological and other problems.

The liquid under test is sprayed into an oxy-acetylene flame through a specially designed injector and a photograph of the flame spectrum is obtained. It is claimed that as little as 0.13 milligram can be detected by this method.

3. " Pastille " method.

In this method a measured quantity of the liquid under test is mixed with powdered graphite or carbon, and the mixture is then dried and

[1] Ramage, *Nature*, 126, p 279, Aug. 23, 1930

[2] Ramage, *Nature*, 123, pp. 601-2, April 20, 1929

[3] *Die Quantitative Spektralanalyse der Elemente*—G. Fischer, Jena, 1929.

pressed into the form of a " pastille," which serves as an electrode for a condensed spark. The other electrode is a small rod of gold. This technique was devised by K. Kellerman,[1] in order to combine the advantages of solid electrodes and the homogeneity of solutions, and was successfully applied to the determination of alloying constituents in steels. There seems to be no reason why this method should not be equally applicable to the analysis of other fluids.

Equipment.

The *Equipment required* should be chosen according to the scope of the investigations contemplated.

(*a*) *The detection of metals by emission spectroscopy* (visual).

To consider first the equipment for the detection of metals by **emission spectroscopy** the first consideration is whether visual or photographic apparatus will be employed. The wider range of possibilities and the more critical character of work done with the latter endow it with unquestioned superiority ; nevertheless the former is so very convenient to use, and it is capable of such delicacy in performance, that with but little experience one may obtain results of a high order of refinement. In considering, for instance, forensic toxicology when the investigator is faced with substances entirely unknown, the quartz spectrograph must be preferred ; but where either the nature of the element to be sought is known, or a fair quantity of material is available so as to provide sufficient scope for wider experimenting in search of the unknown substances, a visual instrument will usually be found adequate, save only for certain elements, notably arsenic and antimony, which give no lines in the visible regions of the spectrum. For the special mercury test described above a visual instrument is to be preferred.

Suggestions for the manner of using any instrument for particular purposes will be made by the Publishers in response to inquiry. The most generally useful *visual instrument* is a Hilger Wave-length spectrometer of the constant deviation type, D 78.

On setting the instrument to the wave-length of an ultimate ray of the element to be sought and observing the spectrum in the eyepiece while the material is brought to incandescence in front of the slit of the spectroscope, exceedingly small quantities may be identified with certainty ; conversely, the non-appearance of the expected line or lines is very strong evidence of the absence of the substance in question. Used in this way it is scarcely inferior in delicacy to the photographic apparatus, while the demonstrations are even more convincing and more readily appreciated

[1] *Archiv fur das Eisenhuttenwesen :* Sept. 1929, Group E. No. 76.

by those who are unable or disinclined to give more than casual attention to the subject of spectroscopy.

A description of a technique and a schedule of equipment applicable to visual analysis by emission spectra, with tables of wave-lengths, will be found in *Visual Lines for Spectrum Analysis*, D. M. Smith (Adam Hilger, Ltd.).

APPENDIX IV

ABSORPTION IN A DIFFUSING MEDIUM

This Appendix has been specially written for this book by Mr. O. W. Pineo to describe proposals for control analysis and prediction of colour in industrial processes, particularly in the dyeing of textiles.

WHILE the spectrophotometric transmission of a mixture of dyes in solution can be predicted from the properties of the separate dyes and solvent (by the application of the Lambert-Beer law) it has hitherto been impossible to predict the spectrophotometric properties of a diffusing medium—for instance, dyed fabric, printed paper, etc.—from the properties of the absorbing materials and the medium. Unpublished work by Pineo [1] shows the Lambert-Beer law to be a special form of a very general law applicable to all manifestations of light absorption, whether through transmission measurements on a non-diffusing medium such as a solution, reflection measurements on a diffusing medium such as a fabric or paint, or measurements on complex laminar, or even inhomogeneous, media such as are met with in colour-printing and colour-photography.

Quite independently of how such presence may be manifested, the presence of absorbing materials in a medium gives rise to a *specific property of the medium* which depends on the specific properties of the separate absorbing materials and the amounts in which they are present *always in the same way,* as dictated by the independent action of the separate materials in the absence of chemical interaction. This specific absorbing property of the medium is, in turn, made manifest in a wide variety of ways corresponding to different measurements made on the medium, and, in the case of a particular measurement, is in a way determined by macroscopic,

[1] Recently at the Massachusetts Institute of Technology, Department of Physics, as a Senior Fellow of the Textile Foundation.

geometrical and other considerations of the particular medium and measurement.

It is well known[1] that if transmission measurements on a given absorbing material in solution are plotted to the proper non-linear transmission scale, there results for different concentrations of the material a family of curves each of which has the same shape *independent of concentration*, and whose position up and down the plot is a measure of its corresponding concentration. In the light of the above paragraph, it is now recognised that this " characteristic colour-curve " is an expression of the specific absorbing properties of the absorbing material, and hence not only invariant with concentration but also with its mode of manifestation whether in a transmitting or diffusely reflecting medium for example, with the macroscopic features of the medium such as length of cell or surface glossiness for example, or with the microscopic features of the medium (whether water or wool, for example). Moreover, the composition of the " characteristic curves " of component absorbing materials to obtain the curve of a mixture is according to a simple additive rule, which preserves the essential identity of the component materials and allows a mixture to be analysed into components. The form of the proper non-linear scale of transmission, reflecting power, etc., is determined by the particulars of the medium and measurement : for the case of transmission t of solutions it has uniform intervals of log log $1/t$; for the reflectance (total reflecting power) r of thick diffusing media—fabrics, ceramic materials, paints, etc. —it has uniform intervals of log $(1-r)/(r-s)$ where s is the reflectance due to surface glossiness alone ; and similarly for other media and measurements.

During the past year Mr. Pineo has been in England where, in conjunction with Adam Hilger, Ltd., he has been engaged in reducing these theoretical concepts to a practical routine for the control, analysis, and prediction of industrial colour processes, particularly dye manufacture and textile application.[2] An improved recording spectrophotometer of the type described by Hardy [3] plots measurements on coloured specimens in a fundamental form, allowing a convenient graphical correlation between the physical colour-properties of the specimens and the colouring processes by which they were produced or could be " matched." On the other hand, the visual properties of the specimens are rapidly determined by a mechanical integraph which performs on their spectrophotometric specifications a mathematical process analagous to colour-vision according to

[1] Geiger & Scheel, *Handbuch der Physik*, vol. xix, p. 665 (1928).

[2] Patents applied for.

[3] Hardy, *J.O.S.A.*, vol. 18, p. 96 (1929).

internationally standardized data[1] on illuminants and normal vision. It is hoped that the insight into colouring processes furnished by the spectrophotometer, and the quantitative results of "mathematical" colour-vision will go far toward placing on a practical scientific basis the present empirical methods of colour industries.

APPENDIX V

THE OSCILLATING SPARK AND ITS CONTROL

The spark circuit in general use for spectrographic work is indicated in the following diagram:

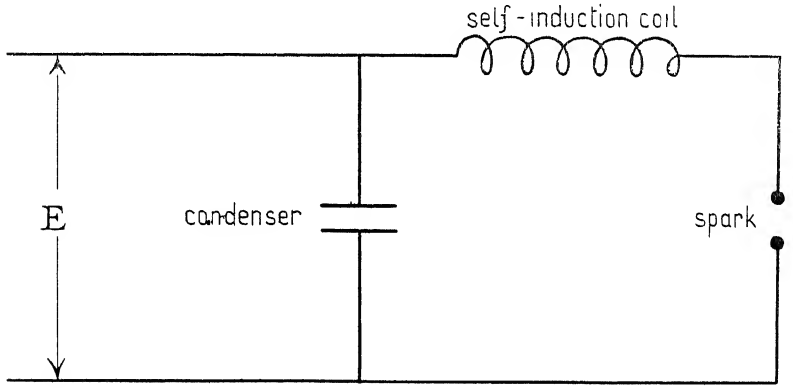


FIG. 45.

E represents the voltage given by the high tension side of a small alternating current transformer. The voltage E of the transformer rises and falls with the same periodicity as that of the generator from which the transformer is fed—say 50 to 80 periods per second. On its rise it charges the condenser up to such a voltage that the dielectric (air) in the spark gap breaks down and the condenser then discharges through the spark gap.

A full analysis of the action of the oscillating spark as it is used in spectrographic work would be very complicated owing to lack of

[1] Smith & Guild, *The C I.E. Colorimetric Standards and their Use. Opt. Soc. Trans.*, vol 33, p 102 (1931-32). Judd, *The 1931 I.C I. Standard Observer and Co-ordinate System for Colorimetry. J.O.S.A.*, vol. 23, p. 359 (1933).

information concerning the relation of the potential difference between the sparking electrodes and the current resulting therefrom, since the spark gap cannot be regarded as having a constant resistance, but there are simple principles and facts which can guide us in obtaining the kind of spark required for any particular purpose.

The standard Hilger sparking set (F 281, F 282, F 283 and F 285), when supplied for an electric supply of about fifty periods per second, has a transformer giving secondary voltages up to 15,000 and the condenser has a capacity of 0·005 mf. The purpose served by the self-induction coil is the removal of air lines, which is sometimes advantageous in spectrum analysis by emission spectra. In absorption work, however, the self-induction coil is omitted since the air lines are no disadvantage—indeed the more lines the better.

For absorption work a spark gap of about 3 mm is used. To start a spark across a gap of this width requires 4,000 volts, but once the spark is passing the voltage required to maintain it is only about 800 volts. What occurs then is that the secondary voltage rises to a point when a spark passes, and the condenser discharges. The voltage E continues to rise (for it has only traversed the small part of its period necessary to reach the sparking potential of 800 volts) until the potential has risen once more to sparking point, when again a spark passes, this process being repeated.

This explains why it is that on observation in a mirror rotating synchronously with the electric supply, a number of sparks (at least 20 or 30) are seen to occur during each period of the supply. Doubtless each spark consists of an evanescent, oscillatory discharge, but it appears in the rotating mirror as a separate flash, separated from the next by a relatively wide dark space. This dark space represents the time required for the transformer to charge the condenser once more to the necessary 800 volts required to repeat the spark. This length of time is in part required for the voltage of the transformer to rise to the required value, but is augmented by the time required for the charging of the condenser.

The outfit as described is admirably suited for ordinary spectrographic work, but it may be that for some purpose considerably greater average intensity of illumination is needed. This can be produced very simply by putting two or more similar transformers in parallel, for with a single transformer the resistance of the secondary is sufficient to cause considerable lag between the voltage at the terminals of the condenser and that which would be produced by the transformer on open circuit. Improvement in the average intensity of the spark (due to the production of a larger number of the flashes) can in this way be effected up to the stage of using three transformers in parallel (or a single transformer of $\frac{3}{4}$ K.V.A. instead of $\frac{1}{4}$ K.V.A.)

Increasing the kilowattage of the transformer beyond this point has no material effect with a condenser of capacity 0·005 mf. and, if still further intensity is required, the capacity of the condenser must be increased so that the quantity of electricity passing in each oscillatory discharge is increased.

On increasing the capacity of the condenser, we shall reach a point when the spark becomes irregular, and this indicates the necessity of further power in the transformer. By increasing the capacity of the transformer and of the condenser alternately in this way, we can increase the intensity of the spark to any extent that may be required.

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